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VOLUME X DECEMBER, 1923 NOS. 3 & 4

ON FIBRES OF "INTERMEDIATE" CHARACTER FOUND IN THE FLEECE OF *OVIS VIGNEI*

BY F. A. E. CREW AND J. S. S. BLYTH.

(*From the Animal Breeding Research Dept., University of Edinburgh.*)

(With Plates XVI and XVII and 3 Text-figures.)

IN an endeavour to define standards to which the fibres of the fleece of the domesticated sheep might be compared, one of us a year ago gave a brief description of a sample of the fleece of *Ovis ammon poli*. In this two sorts of fibre were present, *e.g.* "hair" and "wool," constantly and markedly distinct one from the other in their characterisation: no fibres showing any intermediate characterisation were found. But since that time we have examined a great number of samples of the fleeces of different modern domesticated breeds and consequently it was decided to make a further examination of the fleece of the wild species in order to find out whether in their fleece, as is commonly the case in the domesticated sheep, there is an "intermediate" fibre present.

To Professor Cossar Ewart, who was good enough to supply us with abundant material, we wish to express our thanks.

It was found that two samples, from *Ovis vignei* and *Ovis musimon*, differed from the rest in that instead of being composed entirely of two distinct types of fibre—"hair" and "wool"—they contained a few fibres the characters of which appeared to be intermediate between these two main groups. A detailed examination of the sample from *Ovis vignei* has been made and the description is as follows:

Ovis vignei (Plate XVI, fig. 1).

To the naked eye the sample appeared to be composed of the same two types of fibre found in the fleece of *Ovis ammon poli* and described previously: "hair"—thick, straight, stiffly waved, dead white in its proximal portion and creamy brown in its distal third; and "wool"—fine, springy, curly and of a pearly colour with occasionally a brownish distal half. The wool varied in length considerably, the hair but slightly. Careful examination revealed the presence of fibres of intermediate

character. These fibres could be divided into two classes: (*a*) those which looked like hair but were shorter, less stiff, distinctly finer, and always pointed; and (*b*) those which were indistinguishable from wool in their fine, curly, proximal part, but showed a stiff, straight distal portion, white or pale brown in colour.

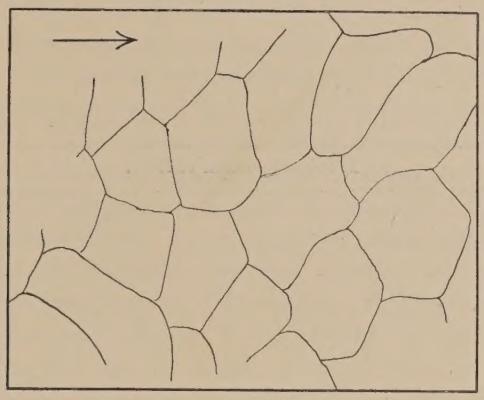
Microscopical Examination.

Wool (Plate XVII, fig. 7). These fibres were exceedingly fine in diameter, usually non-medullated in the proximal part, the shaft being composed, apparently, of cortex merging very near the surface into cuticle; the presence of the latter was indicated solely by the free edges of its external scales each of which encircled the entire circumference of the fibre. Tips were present in all fibres. Towards the tip the fibre broadened slightly and in a few short rod-like areas of air-filled medulla with no definite structure were seen. One or two fibres were pigmented throughout their entire length and a few others were lightly pigmented in the distal portion.

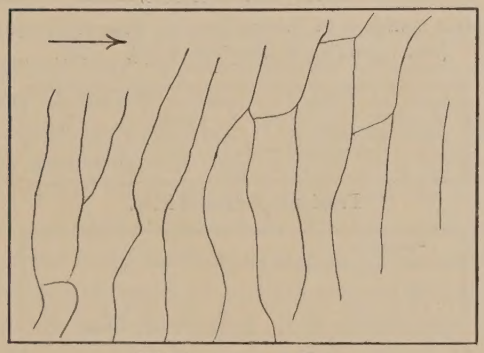
Hair (Plate XVII, figs. 4 and 6). The difference in diameter between the hair and the wool was very marked, that of the former being roughly fifteen times that of the latter. Unlike the wool, the hair had a diameter practically constant throughout the entire length of the fibre save at either end where it diminished slightly. The shaft of the hair consisted of a reticulate air-filled medulla surrounded by an exceedingly thin homogeneous layer identified as cuticle and cortex (*vide infra*) which, in the proximal portion of the fibre, showed slight notches on its outer surface. In the distal portion the place of the notches was taken by projecting scale-like points. In contradistinction to the wool and the "intermediate" fibre none of the hairs had intact tips but a few were practically complete proximally. In these it was found that the fibre narrowed and the medulla gradually diminished and finally disappeared, while the homogeneous layer increased to form the entire diameter of the fibre near the root. All the fibres of this kind were more or less free from pigment in their proximal portion, but distally pigment granules were present in increasing number towards the tip.

In the proximal part of the fibre the surface markings (Plate XVI, fig. 2) were reticulate, resembling very much those of the medulla (Plate XVI, fig. 3) and differing from them only in that while the medullary markings were thicker and coarser and the lines fused forming rough diamonds or irregular triangles, the more superficial markings were extremely delicate, being, in fact, invisible when anything but a $\frac{1}{12}$th oil-immersion was used, and ran together to form well defined clean-cut angles. Distally this

type of surface marking merged into another which took the form of lines roughly parallel to one another, which ran unevenly across the fibre at right angles to its length and, when compared with typical wool surface markings, lay relatively closer to one another. In this part of the fibre the surface consisted of overlapping scales and their ectal edges projected on the lateral margin. Unlike the wool in which but one scale encircled



Proximal portion of fibre.



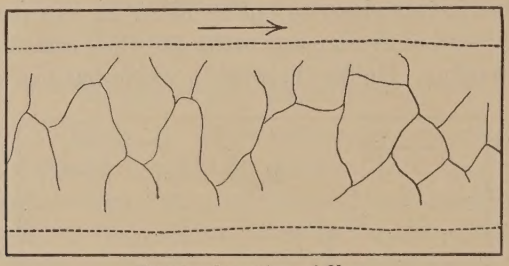
Distal portion of fibre.

Fig. 1. Surface markings on hair of *O. vignei Indicus* (arrow pointing in the direction of the tip of the fibre). ×350.

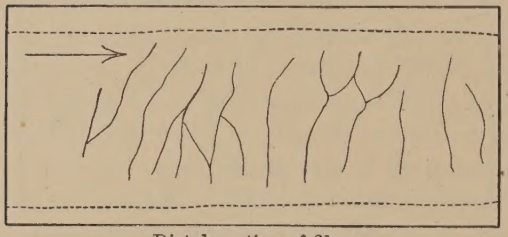
the entire circumference there were here two to four scales. In the fibres which still retained the root it was seen that in this region distinct wool surface markings were present stretching from side to side and ending laterally in free projecting scales.

Intermediate fibres. (*a*) Those which on superficial examination resembled hair had much in common with it when examined micro-

scopically. Fairly uniform in diameter, the medulla of this type was still very large while the homogeneous area surrounding it was relatively thicker. Distally where the fibre began to taper the medulla diminished

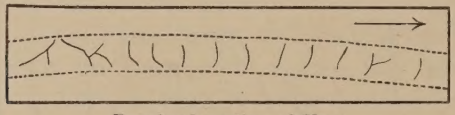


Proximal portion of fibre.

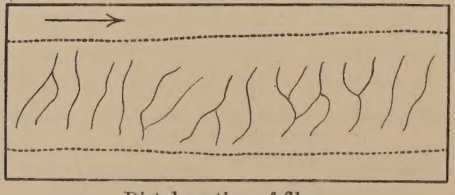


Distal portion of fibre.

Fig. 2. Surface markings on Intermediate (*a*) fibre of *O. vignei Indicus* (arrow pointing in direction of tip). ×350.



Proximal portion of fibre.



Distal portion of fibre.

Fig. 3. Surface markings on Intermediate (*b*) fibre of *O. vignei Indicus* (arrow pointing in direction of tip). ×350.

in size and finally disappeared leaving the homogeneous layer to form a fine tip resembling that of wool. On the surface the lateral edges showed free projecting scale tips throughout except towards the proximal end of some fibres where only notches were to be seen. The surface markings

were like those of the hair. A few of the fibres possessed a fine non-medullated root portion.

(*b*) In those fibres which resembled wool rather than hair, the proximal portion was very similar to wool microscopically. The medulla, however, appeared as rod-like pieces which in the distal portion of the fibre became continuous and widening, as the fibre itself increased in diameter to assume the appearance of the finer fibres of the previous intermediate type (*a*), becoming reticulate and forming a great part of the shaft of the fibre with only a narrow homogeneous superficial layer. The surface markings throughout appeared to be similar to those of typical wool.

In the wool and finer intermediate fibres it was possible to identify cuticle, cortex and medulla, although it was impossible to draw a sharp line of demarcation between these parts. In the case of the hair and the thicker intermediate fibres, however, examination after the ordinary methods of treatment failed to convince us of the presence of cortex. It was found, however, that if, after washing, the fibre was mounted in sulphuric acid and gently heated it was possible by pushing the cover-glass gently along the slide to slough off layers from the surface of the fibre; in some fibres so treated the cuticle came off in large extremely transparent sheets, showing clearly the surface markings. Between this and the main shaft another clear layer appeared showing no transverse markings but having very faint longitudinal striations along which, on further movement of the cover-glass, it tended to break up. The rest of the fibre showed medullary markings. The central layer which was taken to be cortex was more definitely seen in fibres from species with heavily pigmented fleece (Plate XVII, fig. 8) since in these the pigment granules would appear to outline the cortical cells. The homogeneous part at both ends of the fibres was found to fray out into long thin rods similar to this central layer and may therefore be concluded to be also cortex. Ordinary commercial sulphuric acid was found more satisfactory for our purpose than a dilute solution, as the latter seemed to cause more thorough disintegration, cells coming off singly instead of in layers.

The surface markings of the hair fibres are not corticular, as was suggested in the description of the fleece of *Ovis ammon poli*: they are either medullary or cuticular.

As to the significance of the intermediate fibre: it cannot be regarded as an undeveloped hair, since it has been shown that hair growing, as it does, during summer is more or less uniform in length in the winter-coat. The fact that a few of these fibres were complete and therefore ready to shed also supports the evidence against this contention. There is no

suggestion that the intermediate fibre is the common type from which both hair and wool later develop: the two types of intermediate fibres found in the fleece of *Ovis vignei* are constantly distinct. One (type *a*) is always uniform in diameter, the other (type *b*) always thicker in the distal than in the proximal portion; there is no evidence of any transformation of the one type into the other. On the strength of that it would seem improbable that the intermediate type is a modification of only one type of fibre—only hair or only wool—though it would seem possible that it is a modification of both.

Table I. *O. vignei.* Variation in the Diameter of Fibres* (Centimetres).

	Intermediate fibres		
Hair	*a*	*b*	Wool
0·0919	0·0323	0·008281	0·005733
0·0864	0·0266	0·009018	0·005733
0·0946	0·0583	0·009018	0·005642
0·0915	0·0505	0·009018	0·005460
0·0925	0·0226	0·009100	0·005460
0·0939	Mean =	Mean =	0·005278
0·0965	0·0381 ± 0·0042	0·008887 ± 0·00008	0·005187
0·0901			0·005187
0·0825			0·005005
0·0873			0·005005
0·0826			0·004914
0·0958			0·004823
0·0941			0·004277
0·1006			0·004095
0·0987			0·003913
Mean =			Mean =
0·0916 ± 0·0009			0·005047 ± 0·000095

* Measurements of diameters were taken in the proximal half-inch of the fibre.

Table II. *O. vignei.* Variation in Length of Fibres.

		Intermediate type		
	Hair	*a*	*b*	Wool
No. of fibres in sample	50	14	15	236
Shortest fibre... ...	3·3	2·3	2·05	0·65
Longest fibre... ...	3·8	3·5	2·8	3·55

DESCRIPTION OF PLATES XVI AND XVII

Fig. 1. Sample of fleece of *Ovis vignei Indicus.* ×2.
Fig. 2. Cuticular markings on hair. ×700.
Fig. 3. Medullary markings on hair (same field). ×700.
Fig. 4. Longitudinal section of hair. ×105. Left section near lower surface showing cortex below and medulla above.
Fig. 5. Longitudinal section of intermediate fibre. ×105.
Fig. 6. Cross-sections: above—thick intermediate fibre; below—hair. ×105.
Fig. 7. Wool of *Ovis vignei*, optical section. ×105.
Fig. 8. Portion of hair from *O. musimon*, treated with sulphuric acid. ×200.
ct = cuticle; *cr* = cortex; *m* = main shaft of fibre showing medulla.

(*Received May 21st*, 1923.)

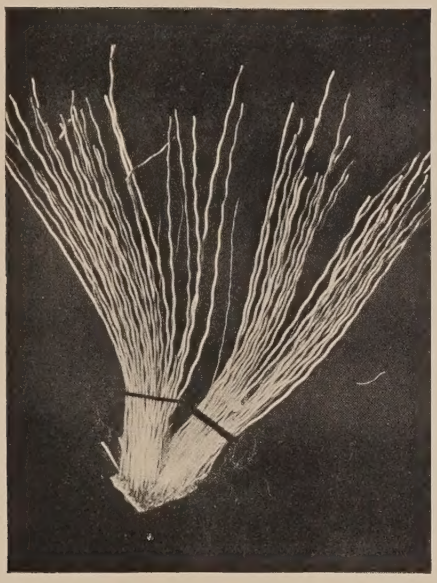


Fig. 1.

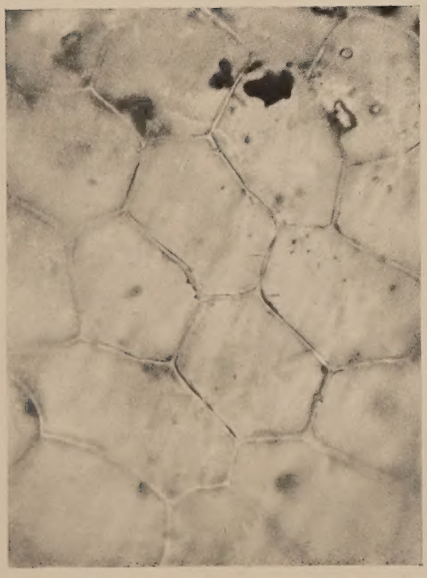


Fig. 2.

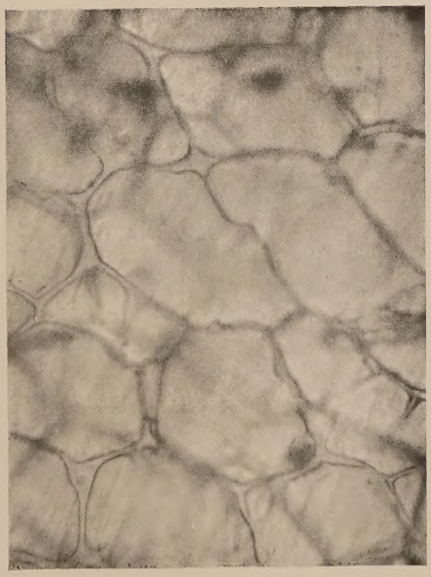


Fig. 3.

CREW & BLYTH.—On Fibres of "Intermediate" Character found in the Fleece of *Ovis vignei*. (pp. 295—300.)

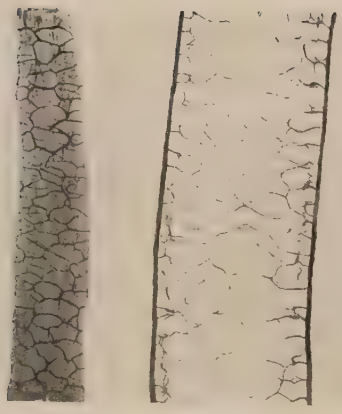


Fig. 4.

Fig. 5.

Fig. 6.

Fig. 7.

Fig. 8.

CREW & BLYTH.—On Fibres of "Intermediate" Character found in the Fleece of *Ovis vignei*. (pp. 295—300.)

MICROLOGICAL ANALYSIS OF TWO FLEECES FROM BLACKFACE SHEEP

By JANET S. S. BLYTH, B.Sc.

(*From the Animal Breeding Research Department, University of Edinburgh.*)

(With Plate XVIII and 4 Text-figures.)

Partly on account of the wide distribution of the Blackface Mountain Sheep and partly owing to the practice of confining to each hill-grazing its own indigenous stock, there is, as might be expected, a wide variation in the type of fleeces from the various flocks of this breed. It being, therefore, quite impossible to take any particular fleece as typical of the breed, a skin and a fleece were used for the purpose of this paper. The skin (1) was from a show ram which had died in September, and, having missed the summer clip, the fleece was longer than usual. In appearance it was of the strong "hairy" quality commonly seen in the showyard sheep except for a small patch on the right shoulder where the long hair fibres had almost entirely given way to the much finer fibres (wool). In this patch the undercoat was visibly coarser than when completely covered by the long outer coat as was the case throughout the rest of the fleece. Kemp was found all over the fleece especially in the hind quarters.

The fleece (2) was the ordinary commercial type cut—it is understood—at the usual time. It was somewhat "open"; the hair fibres were much finer than in the previous case and did not by any means cover up the woolly undercoat although longer and greater in calibre. Only a very little kemp was present in the forequarters but it was very abundant in the hindquarters.

Samples taken from the shoulder and britch showed that the fibres could be classed into three types:

Easily detected by the contrast which its dead-white colour makes against the creamy brown colour of the rest of the fleece the first type, kemp, is found in considerable quantity throughout fleece (1). It is very brittle, many fibres having a number of "breaks" or bruised parts along their length and they usually have a peculiar stiff waviness. Shorter than

the other constituents of the fleece kemp varies from ·75 inch to six inches, with most of the fibres about four inches long; all end in a fine tip except those which, very much bruised and broken, lie at different angles to the general direction of the fleece. Most of the longer fibres are loose and unattached to the skin, lying in the "matt" formed by the other two types, with their proximal ends, which taper down finely to terminate in a slight bulb, lying slightly nearer the skin than the fine portion in the next type (*v. infra*). The remaining fibres still attached to the skin do not possess a fine proximal portion except for one or two of an obviously pathological nature, which are brown, shrunk, twisted, and flattened for most of their length.

The second and longest type of fibre, hair, in fleece (1) were coarse and straight except where most had a single loop or waved part about three inches from the proximal end. They overlapped by several inches the other two types. Nearly all were more or less uniform in length, only a few having tips and these were either slightly shorter than the average (which terminate in a brown, much worn part) or else about three to four inches long when they were difficult to distinguish from kemp except by the latter's greater calibre and stiff waviness. About three inches from the proximal end (*i.e.* in the region where the looping occurs) a slight thinning could be discerned on the majority of fibres. Occasional loose fibres were found and were generally complete proximally with the root as in the case of kemp lying in the region of the loop on the other fibres.

In this fleece there is a distinct gap between this type and the next which comprises the finer shorter fibres of the woolly undercoat. For the most part these are curly with straighter, thicker distal parts either ending in a loosely waved portion or having the most distal half inch of their length straight. Tips are only found in one or two cases. A very few extra long fibres resemble the average distal portion throughout their whole length; on the other hand some fibres shorter than the rest are seen to be curly throughout, and among these only a few tips are present. Loose fibres occur; these are generally complete and as in the case of the other loose fibres they are shorter proximally ending in the region of the thin part of the hair.

Pigmented kemp fibres were found scattered throughout the whole of the fleece but coloured fibres of the other two types only occurred in small patches on the edge of the fleece.

In fleece (2) the three types of fibre present in fleece (1) are also found but with the following differences.

Kemp is only occasionally found as a complete fibre—that is the tapering proximal portion is generally absent. Those which are complete, however, terminate close to the cut edge of the fleece.

The long hair fibres are finer than in fleece (1) and while they are on the average distinctly longer than the wool the difference between the groups is less marked, one or two fibres being found which cannot be definitely allocated to either group. There is no loop on the fibres and individual fibres seem fairly uniform in bore throughout their length except at the proximal end where they are as a rule finer. Many of the fibres are straight and only slightly curved; others slightly finer have their proximal half very loosely curled.

Wool fibres appear to be much as described in fleece (1) with the exception that tips which are only occasionally present are found only among the longer fibres. The few complete fibres of both hair and wool which are present are found to terminate proximally near the cut edge of the fleece.

Pigmented fibres formed here and there throughout this fleece were found to be of all three types.

MICROSCOPICAL EXAMINATION.

For the purpose of microscopical examination numerous fibres were treated in a number of different ways according to the results desired. In all cases the fibres were first washed in methylated ether to remove the grease and foreign matter with which they are coated. By staining in picroformol and mounting in balsam a general view of the fibre in optical section was obtained. A series of fibres so treated were used for the preparation of the table of diameters (Table III). Longitudinal and cross-sections were obtained, after staining with picroformol, by embedding in paraffin wax and keeping the blocks in water for, at least, a week before cutting. To bring out the surface markings more clearly Hausman's method of dipping the fibre in an alcoholic stain was adopted. Solutions of various stains were used differing in strength both as regards alcohol and stain, but in all cases the results were irregular. Gentian violet and eosin appeared to give the best results. To ascertain in doubtful cases whether cortex and cuticle were present fibres were treated in sulphuric acid.

Kemp (Plate XVIII, fig. 1). The proximal portion is found to consist of an apparently homogeneous cortex, non-medullated, comparatively fine, which is fairly uniform in diameter throughout its (short) length. Here and there small black marks are seen dotted over it and towards the end

these are very numerous, giving the appearance of pepper shaken from a pot. On careful focussing, they are seen to be tiny air-filled holes which lie not only on the surface but apparently all through the shaft. The end itself is frayed into hundreds of tiny narrow rod-like pieces and projecting ectally with their free ends pointing towards the skin.

Travelling distally the homogeneous portion broadens, a black (air-filled) rod-like medulla, occasionally discontinuous at first, appears and growing in breadth much more rapidly than the fibre itself soon fills almost the entire shaft, the homogeneous portion being represented in longitudinal section by a very narrow band running up each lateral edge. Soon after the medulla begins to broaden, its structure is indicated by the manner in which the air appears to lie in a collection of partly fused bubbles and where in some fibres the air has been expelled by the balsam the reticulate spaces typical of a broad medulla are easily seen stretching from side to side of the medulla and showing several layers deep with occasional septae hanging between. Cross-sections also show the medullary network broken up by torn septae. The width of cortex in individual sections is always fairly uniform.

This picture now holds good for the main length of the fibre with exceptions that "breaks" occur at intervals. At these the regular pattern of the medulla becomes confused and usually the lateral edges of the hair jut out in two distinct points. The fibre remains fairly uniform in bore until more than half its length has been passed over; after that it decreases very slowly in size till about half an inch from the distal end it rapidly tapers out to a fine tip, the medulla thinning out more rapidly than the rest of the fibre, so that it ends half way to the end while the cortex increases in size, filling the whole shaft and then thins slowly to form the long fine tip.

On the lateral edges of the fibre a little way from the frayed proximal end free protruding edges of scales are seen in longitudinal section. Travelling distally these are followed at small intervals by similar scales until the fibre has attained its full width where they appear to be gradually replaced by vaguely raised ectal portions or notches. This holds until in the distal half notches are again replaced by projecting scales which are seen right out to the extreme tip of the hair.

An examination after treatment for surface markings (Fig. 4) showed that these were of various types and corresponded but roughly with the different portions of the fibre. The most proximal markings (*i.e.* those over the homogeneous part of the shaft) run irregularly across the fibre from one side to the other, roughly parallel but occasionally

bending to run into one another, forming a series of single overlapping scales as in typical wool. These gradually, as the fibre attains its full width, merge into another distinct type of markings which are of reticulate appearance; the meshes being four or five sided and roughly square, they generally lie diagonally to the length of the hair (*i.e.* with a corner pointing distally). Further on these broadening gradually merge into another type usually found in the distal half where the fibre is slightly thinner. These are very broad shallow scales and one or two to the breadth of the fibre may be seen. Finally, these merge into the markings of the tip where each scale more or less surrounds the circumference of the fibre. The edge of these scales shows up as an exceedingly irregular and jagged line running unevenly across the fibre.

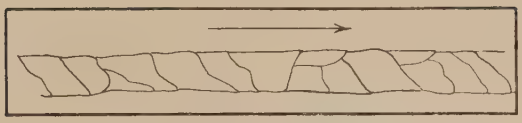
Hair (Plate XVIII, figs. 2 and 3). Individual fibres of this type showed much more variation in calibre than in the last. The air-filled medulla, reticulate throughout and still large in the majority of cases, is smaller and the cortex broader than in the case of kemp. In the finer fibres, as also in the thin portions of the others, the medulla usually thins down considerably and may appear to lose its structure showing as a solid rod—continuous or discontinuous, while the cortex may broaden slightly at the expense of the medulla. The cortex is of homogeneous appearance in this type except in a few cases where, at the distal ends, it has been split up through weathering into rod-like pieces much as in the proximal end of the complete kemp fibre, but here we have a full-size medulla present right to the end and fraying is not so irregular. The cortex in longitudinal section is represented by two narrow bands, one lying up either side of the medulla. These are often of unequal width and this is also borne out in cross-sections where it is seen that, as a rule, the cortex is thicker on one side of the fibre than on the other. While some of the complete fibres ended proximally in a short non-medullate portion running out to end in a bulb of cortex frayed up into separate cells, others had the portion covered by a shrunken twisted sac, apparently the hair follicle.

Surface Markings (Fig. 3). On the thickest part of the fibres the markings tend to be like those of kemp at its widest part; generally, however, their length lies along the direction of the hair or at a slight angle to it rather than across the breadth. As the hair narrows this lengthening is more obvious, but the appearance of overlapping scales which can be seen in the first case is less obvious here and, in fact, the contour of the scales would often lead one to believe that they overlapped in the opposite direction, did not those on the lateral margin of the hair with their ectal edges pointing distally belie this. Very fine portions of

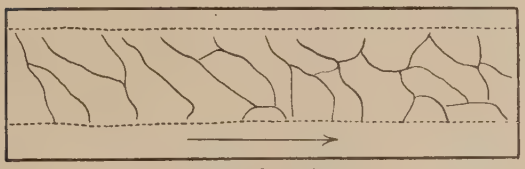
fibres of this type such as shown by some fibres at the "nick" (or thin portion) show a further lengthening of the scales; these lie diagonally across the fibre in the main tending slightly to lie parallel with its breadth. Although in some cases lying at right angles to the length these markings are never at any time like those in the distal half of the kemp fibre, since the impression of regularity obtained from the latter is never given here.

Where tips were present these were formed as in the case of kemp, the homogeneous cortex running out to the tip surrounded by cuticular scales, each sitting in the cup of the other as in typical wool. It was noted that in many of these fibres the distal end, when treated for surface markings, presents a false picture in that, being very translucent and having lost most of its cuticle by attrition and other weathering processes to which it has been subjected, the medulla shows up as if it were on the surface and may be mistaken for cuticular markings. Especially is this the case when eosin has been used, as it is readily picked up by the medulla.

Wool (Plate XVIII, figs. 4, 5 and 6). This varies in calibre both within its type and within individual fibres. The fibres in fleece (1) are thick for about an inch of their length proximally, then thin down and, remaining fine for a considerable part of their length, they thicken again till the straighter distal portion is reached; this latter part is fairly uniform in diameter. In fleece (2) no thick proximal part is found. The thickest parts of this type of fibre are as thick and in occasional cases thicker than the finest portions of "hair"; also they approach these in structure, possessing an air-filled rod-like medulla and a comparatively thick cortex covered by projecting cuticular scales. In optical section the medulla shows no structure but longitudinal and cross-sections show, although somewhat vaguely, that an arrangement of cells is present. Apparently these are placed regularly one above the other and in diameter the medulla may be one or two cells deep. Throughout the thick portion it is in the main continuous, being discontinuous during the transition from the thick to the thin portion of the fibre. Here depending on the average size of the fibre it may either disappear altogether or fragments of medulla may appear irregularly down the centre of the fibre. Surface markings (Fig. 1) in the finer part, especially if non-medullated, are those of typical wool, *i.e.* one scale completely encircles the circumference of the fibre and each scale sits in the cup formed by the outward and distally projecting edge of the one beneath it. As the fibre widens (Figs. 1 and 2) more than one scale per diameter is seen and these generally tend to stretch irregularly across the fibre with their length diagonally across the shaft, giving much



(*a*) Proximal portion.



(*b*) Distal portion.

Fig. 1. Wool. Surface markings. ×225.

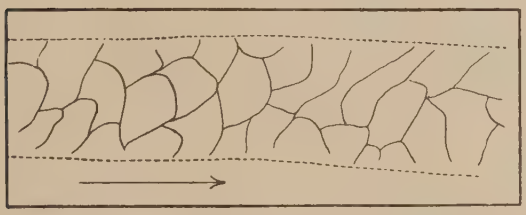
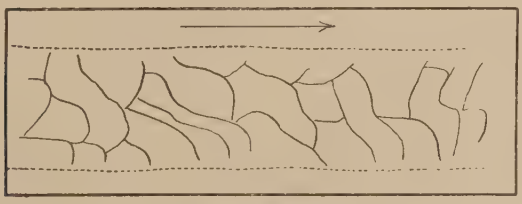
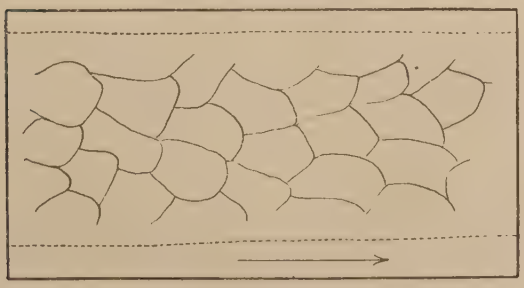


Fig. 2. Wool. Surface markings on thick wool fibre corresponding to Plate XVIII, fig. 4. ×225.

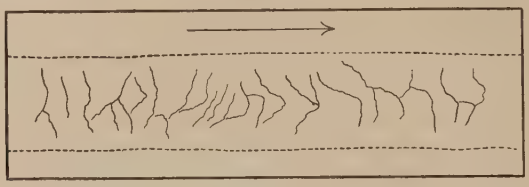


(*a*) Proximal portion.

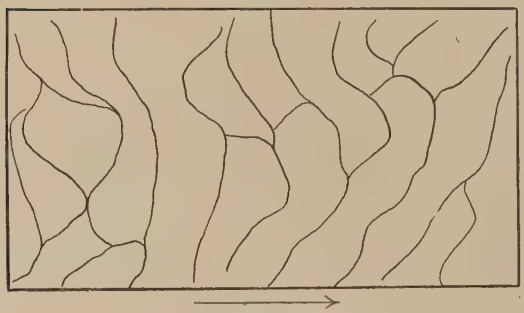


(*b*) Distal portion.

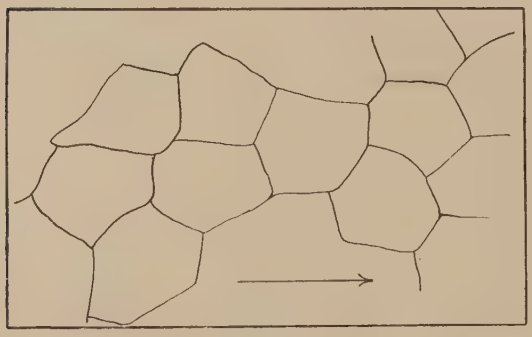
Fig. 3. Hair. Surface markings on the thick and thin portions of a hair fibre. ×225.



(*a*) Tip.



(*b*) Distal portion.



(*c*) Proximal portion.

Fig. 4. Kemp. Surface markings at different points along the kemp fibre. ×375.

the same appearance as is found in the finer portions of the hair type. As in the other types the tip is formed by the clear smooth cortex and the cuticular scales only, the medulla having terminated some distance from the end.

Structurely there is less to define the three classes of fibres found in the Blackface fleece than is given by the general appearance to the naked eye examination. The broad shallow scales (Fig. 4*b*) found on the distal half of kemp seem characteristic of that fibre and do not appear to occur in hair. Cortex and medulla on the other hand may be so similar in these two types as to be useless for their identification. In distinguishing

microscopically between hair and wool the difficulty is still greater; here the cuticle lends no help and one can only say that in wool the proximal third of the fibre is finer whereas in hair only a small part of the proximal end is finer than the rest of the fibre.

Kemp appears to differ from the other two types of fibre in that it is completely shed at least once a year. From this, as from its structure, it seems that kemp is analogous to the hair of the primitive breeds. The "hair" of the Blackface is not hair defined on these standards but in its thicker parts approaches in structure the Intermediate (*a*) type of fibre found in *O. vignei*[1], while the wool varies from typical wool to something which tends to the Intermediate (*b*) type. Both these Blackface types are, of course, much longer than those of the primitive breeds and also differ in that only a few of their fibres are shed annually, the rest showing a finer part in this region which in the case of wool is continued distally a third of the length of the fibre. It may be noted that this "shedding" region corresponds to the "rise" of the fleece which is generally taken as the signal for clipping, so that actually the fleece is sheared at its weakest point.

The looping of the hair fibres which occurs in fleece (1) seems to indicate that the wool stops growing for a time while the hair held in check by the matt of fibres kept close above it by the non-lengthening wool fibres is forced to curl or bend in order to continue its growth. In this the Blackface fleece again shows a resemblance to the primitive breeds where there is no summer growth of wool. Following out this analogy it might be expected that there would also be a period of no growth in the hair and kemp fibres earlier in the season, but whether this occurs is not evident from the present material. "Grey" fibres appear to take their origin from pigmented patches in the skin, since all three types of fibre may be pigmented.

SUMMARY.

The Blackface Mountain sheep carries a very long and coarse fleece consisting of three types of fibre:

(1) *Kemp*, the shortest, coarsest fibre is completely shed annually. It has the structure of typical hair.

(2) *Hair*, the longest type, varies a great deal in diameter. Under normal domesticated conditions it has little or no tendency to curl. Only a small percentage of fibres are shed annually, the others showing a finer

[1] F. A. E. Crew and J. S. S. Blyth. "On Certain Fibres of an Intermediate Nature found in *O. vignei*," *Ann. of Applied Biol.* x, p. 295.

part in the same region. In structure the coarser fibres resemble the Intermediate (*a*) type of *O. Vignei*.

(3) *Wool*, the finest fibre, tends to vary from typical wool structure to that of the Intermediate (*b*) type of *O. Vignei* and is finer in its proximal third than in its distal part. It is always curly more or less according as it is finer or thicker. Only a few fibres are shed annually.

The variation in Blackface fleeces would appear primarily due to the variation in the character of the second type of fibre. "Grey" fibres found scattered throughout the fleece may be of all three types.

Table I.

	Fleece (1)			Fleece (2)	
Hair	Shoulder cm.	Britch cm.	Patch on right shoulder* cm.	Shoulder cm.	Britch cm.
Average length of fibre	41·6	38·5	—	31·2	33·0
Longest fibre	43·9	41·1	—	32·0	35·1
Shortest fibre	37·3	29·5	—	30·2	29·2
Wool					
Average length of fibre	21·87	21·52	20·48	15·95	18·36
Average no. of waves/fibre	47·75	37·75	44·84	30·45	32·33
Average no. of waves/cm.	2·18	1·75	2·19	1·91	1·76
Greatest no. of waves/cm.	3·47	2·43	2·85	2·83	2·76
Longest fibre	30·5	25·9	22·6	20·82	22·86
Shortest fibre	18·55	17·78	18·04	11·7	14·0

* Patch containing practically no hair fibres.

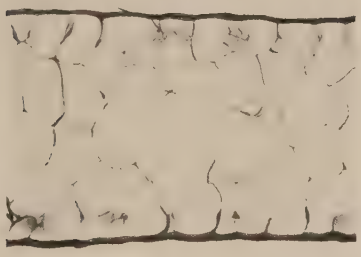
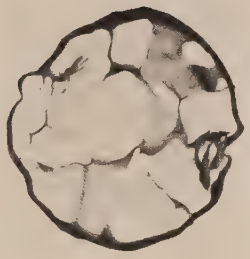
Table II. *Fleece* (1).

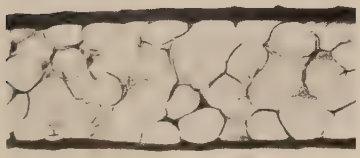
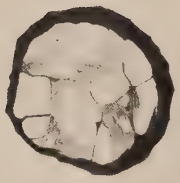
	Hair	Wool	Kemp*	Total	Total per sq. inch
	per $\frac{1}{8}$ square inch				
Shoulder					
No. of fibres	315	960	74	1349	10,792
Percentage of fibres	23·35	71·16	5·49	—	—
Britch					
No. of fibres	417	751	109	1277	10,216
Percentage of fibres	32·65	58·81	8·54	—	—

* Only those kemp fibres which were attached to the skin were counted.

Table III. *Average Diameter.*

Fleece	Hair cm.	Wool cm.
1	·0076	·00277
2	·0058	·00307

1 (*a*).   1 (*b*).

2 (*a*).   2 (*b*).

3 (*a*). 3 (*b*).

4 (*a*). 4 (*b*).

5 (*a*). 5 (*b*).

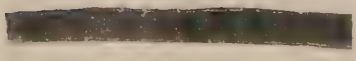
6 (*a*).  6 (*b*).

Table III (*a*).

Giving average diameters at different points along the fibres in both fleeces travelling from tip to root.

Fleece (1)	Fleece (2)
0·0094	0·0063
0·0086	0·0063
0·0061	0·0051
0·0071	—
0·0068	—

DESCRIPTION OF PLATE XVIII

Fig. 1. Kemp, thick fibre. × 200.
Fig. 2. Hair, thick part of medium sized fibre. × 200.
Fig. 3. Hair, thin portion of same fibre. × 200.
Fig. 4. Wool, distal part of one of the thickest fibres from the part of the fleece which was almost free from hair. × 200.
Fig. 5. Wool, distal portion. × 200.
Fig. 6. Wool, proximal portion. × 200.
a, sagittal section; *b*, transverse section.

(*Received June 6th*, 1923.)

CARBON DIOXIDE IN RELATION TO GLASSHOUSE CROPS

PART I.

THE DISTRIBUTION OF CARBON DIOXIDE IN GLASSHOUSE ATMOSPHERES

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(With 2 Text-figures.)

Introduction. Investigations carried out by continental workers during recent years have drawn attention to the possible increases in crop-yield resulting from the artificial enrichment of air with carbon dioxide. The results claimed have raised wide-spread interest in the horticultural world and in the interests of the glasshouse industry in this country it became essential to investigate how far the claims were valid under the conditions prevailing here.

Before attempting to increase the concentration of atmospheric carbon dioxide under glass it was imperative that some information should be available concerning its normal distribution and variation under working conditions. The work dealing with this phase of the problem is described in Part I.

It is generally held that in the open, atmospheric carbon dioxide is distributed in layers, the highest concentration lying near the soil surface. Under glass where manurial treatment is comparatively extravagant one would expect to find this state of things accentuated. It has been claimed that in a cucumber house "the concentration is greatest near the soil surface—nine to fourteen parts per 10,000—and least at the top of the house—four to five parts per 10,000" (1). Beyond this there seems to be no indication that glasshouse atmospheres have been analysed.

As will be shown here, no regular variation in the carbon dioxide concentration can be found at different levels in a commercial glasshouse. For the experiment a cucumber house measuring 100′ × 44′ was used, the other dimensions being shown in Fig. 1. Samples were taken at some or all of the points marked *a* to *m* and analysed.

It was found that the average concentration of carbon dioxide varies during twenty-four hours, showing a maximum in the early morning and a minimum in the early afternoon. An examination of Table I shows

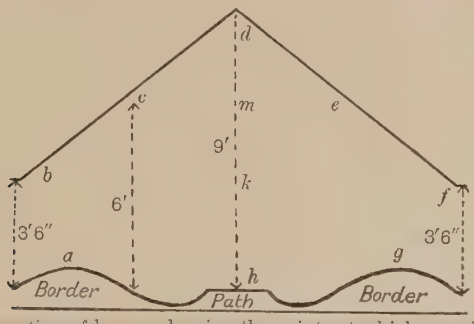

Fig. 1. Side elevation of house, showing the points at which samples were taken.

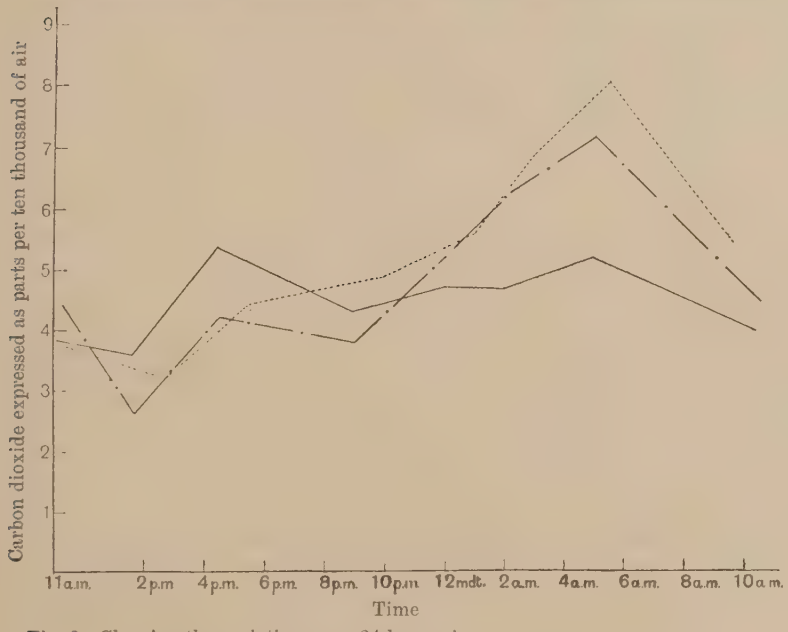

Fig. 2. Showing the variation, over 24 hours, in:
(1) the average concentration of carbon dioxide - - - - - - - - - - -
(2) the concentration at one of the soil surfaces ——• —•——
(3) the concentration at the apex of the house ——— -

that the concentration at definite points varies very irregularly. As already stated, it was expected that a definite stratification of the gas would be found. If this were exactly so then the concentrations at the

Table I. *Observations made during October* 1922[1].

Date 1922	Time	Position									
		a	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*k*	*m*
Oct. 11	9.45 a.m.	3·4	1·5	—	5·8	10·0	7·6	9·0	3·6	6·5	2·7
,,	12.45 p.m.	1·1	3·7	1·8	3·7	1·5	—	4·5	3·0	4·4	5·7
,,	4 ,,	1·3	3·0	5·4	4·7	2·0	4·6	4·2	5·1	1·8	4·5
Oct. 12	11.30 a.m.	1·1	4·2	5·2	4·7	3·6	3·8	7·0	—	3·8	9·4
,,	3.30 p.m.	—	5·2	6·5	4·4	1·7	1·8	1·9	—	—	—
Oct. 13	9 a.m.	7·4	6·7	2·8	5·0	2·0	7·2	9·6	10·0	6·2	—
,,	5 p.m.	1·5	1·8	2·2	2·2	2·0	3·8	3·8	—	—	—
Oct. 16	9.30 ,,	1·2	5·5	2·4	—	9·5	4·7	5·1	6·3	6·8	6·9
,,	12 midnight	4·0	6·7	10·4	—	2·1	2·2	7·9	3·2	6·1	4·6
Oct. 17	3 a.m.	4·3	9·1	7·3	10·5	7·5	8·9	2·0	—	6·3	8·8
,,	6 ,,	3·0	5·7	8·8	8·4	3·0	5·1	7·2	16·1	3·4	5·3
,,	9.30 p.m.	5·1	5·8	4·0	6·3	11·9	4·6	5·3	2·5	3·9	8·0
,,	12 midnight	6·9	4·3	5·4	5·6	7·1	9·6	2·6	6·6	2·5	2·7
Oct. 18	3 a.m.	4·4	8·1	4·1	8·8	7·0	—	5·0	9·0	7·7	5·9
,,	6 ,,	—	6·7	8·1	8·4	6·2	8·8	5·2	7·1	5·0	7·0
,,	9.30 p.m.	5·3	4·0	7·0	3·8	3·8	8·0	5·7	5·9	7·8	4·4
,,	12 midnight	2·0	6·6	7·6	5·0	6·0	—	6·9	4·3	4·0	7·7
Oct. 20	3 a.m.	5·8	7·9	8·3	5·8	8·4	10·0	6·9	5·4	7·1	—
,,	11.15 ,,	3·0	2·7	3·6	4·0	—	—	—	4·6	4·0	5·9
,,	2.45 p.m.	3·8	2·9	2·5	3·6	—	—	—	2·7	4·0	3·5
,,	5.30 ,,	2·0	4·2	5·4	4·6	—	—	—	4·8	4·7	5·7
,,	10 ,,	4·9	5·4	4·7	4·4	—	—	—	3·8	—	4·8
Oct. 21	1 a.m.	5·5	4·4	—	4·9	—	—	—	5·1	7·4	5·7
,,	3 ,,	7·7	7·2	10·2	4·9	—	—	—	6·2	7·7	4·8
,,	5.30 ,,	7·7	8·1	11·2	6·2	—	—	—	7·4	8·8	7·1

positions *b*, *k* and *f* in Fig. 1 and also at *c*, *m*, and *e* would be approximately the same, but as will be seen from Table I, no similarity occurs. In fact, individual variations are most irregular for all positions. While this is so, it is found that the average concentration varies comparatively regularly with time, this being shown in one of the curves in Fig. 2. The irregularities observed are difficult of explanation and at present one can only attribute them to the fact that the air in a glasshouse is never still. As a result, various currents must be set up and these would tend to disturb any localisation of carbon dioxide which might otherwise persist. In addition to this purely mechanical effect the processes of assimilation and respiration of the plants in the house come into play, although these may not produce the abrupt irregularities observed. It is also possible that the methods of sampling or analysis are at fault. Alternative methods of sampling were tried and will be dealt with presently. The actual analyses were carried out in the portable Haldane

[1] Observations given as parts of CO_2 per 10,000 of air.

apparatus, and the Pettenkofer method seems to be the obvious alternative. This method however is hardly justifiable since the large volume of sample required would in itself be sufficient to cause quite an appreciable local disturbance.

Since stratification could not be found under the conditions described it was considered desirable to determine whether it existed under different conditions. The soil of a small empty chamber of a thousand cubic feet capacity was covered with a layer six inches thick of fresh stable manure. It was thought that by this means the carbon dioxide in the air would be increased and any tendency on the part of the gas to stratify would be accentuated. Samples of the air were then taken periodically at heights of 1′ 3″, 3′ 6″, 7′ 6″ above the surface of the manure. Some dozen sets of observations were made, and typical readings are quoted in Table II. It is evident that there is no regular variation at different heights. In case the presence of growing plants might affect the atmospheric distribution the experiments just described were repeated in a cucumber chamber of similar dimensions in the presence of the same amount of manure. Typical results are shown in Table III. It will be observed that compared with Table II the results give lower values for the carbon dioxide. It would seem that this must be attributed to assimilation since all these determinations were made in the day time.

Table II. *The concentration of carbon dioxide in the atmosphere of an empty house containing only soil and fresh stable manure. Capacity approximately* 1000 *cu. ft.*

Height from soil surface	Concentration of carbon dioxide		
	10 a.m.	2 p.m.	4 p.m.
1′ 3″	11·7	5·5	3·2
3′ 6″	15·0	6·1	3·7
5′ 6″	14·7	4·7	3·5
7′ 6″	14·2	7·0	4·0

Table III. *Concentrations in a cucumber house with soil covered with a six inch layer of fresh stable manure. Capacity* 1000 *cu. ft.*

Height from soil surface	Concentration of carbon dioxide			
	10 a.m.	12 noon	2 p.m.	4 p.m.
1′ 3″	4·2	2·5	2·0	1·6
3′ 6″	3·6	2·4	2·4	1·5
5′ 6″	3·2	2·6	2·3	2·0
7′ 6″	3·2	2·5	2·3	2·0

It may be argued that the method of sampling might interfere with the sample taken. It was considered that in either of the two small

chambers already referred to that this objection might be overcome by hanging the sample bottles on nails fixed at the different heights. This was done and the unstoppered bottles put in position about three hours before sampling. At the appointed time the bottles were quickly stoppered, taken out and analysed. Again similar results were obtained and are shown in Table IV.

Table IV. *Concentrations shown when sample bottles were left in position for three hours before analysis. Empty house* 1000 *cu. ft. capacity in which the soil was covered with a six inch layer of fresh stable manure.*

Height from soil surface	Concentration of carbon dioxide		
	10 a.m.	2 p.m.	4 p.m.
1′ 3″	6·1	5·4	5·0
3′ 6″	5·7	3·9	4·6
5′ 6″	6·3	4·4	4·8
7′ 6″	6·1	5·4	4·6

Experimental. The samples were taken in two-ounce bottles fitted with glass stoppers and were filled by aspiration, duplicates being taken simultaneously by means of a T-piece. The samples from positions *b*, *c*, *d*, *e* and *f* (Fig. 1) were taken above the foliage, while those at *a*, *g* and *h* were taken on the soil surface. They were all analysed in duplicate in the portable Haldane apparatus in the prescribed manner (2). In consequence the readings shown in the table are the mean of four determinations and all the results are expressed as parts of carbon dioxide per ten thousand of air.

Alternative methods of sampling. The possibilities with an alternative method of sampling have also been examined. Apart from disturbances caused by the workers in a house, those due to the observer himself would tend to upset the readings. Accordingly it was thought that if samples could be taken from some distance away the difficulty would be overcome. An obvious suggestion is the arrangement of a system of glass tubing of sufficient length to obtain the desired sampling. To be effective the bore of the tubing must be small, otherwise displacement of air in sampling would be sufficient to cause an appreciable disturbance. About ninety feet of capillary tubing was fixed upon suitable supports—one end being open at the sampling position while the other was connected to the tap of a nitrometer. It was intended that mercury from the nitrometer should be forced down the capillary tubing till the latter was full. By altering the level of the mercury it would return and a sample should eventually be drawn into the closed limb of

the nitrometer. The transference of the sample to the Haldane apparatus would then be a comparatively easy matter. Actually, in practice the whole procedure was found to be unsatisfactory. In spite of continual adjustments of the capillary tubing, filling it with mercury was invariably a lengthy and tedious process, and the taking of a sample was accomplished on very few occasions only. Air bubbles continually made their appearance and in spite of many adjustments it was impossible to obtain satisfactory results. After a fortnight this method was abandoned. The alternative was to take a sample from immediately outside the house by which means a much shorter length of capillary tubing would be necessary. This method was at once found to be unsatisfactory owing to the appreciable difference in temperature inside and outside a house.

In spite of the objections which have been noted to methods of sampling it is maintained that if the suggested atmospheric stratification does exist in glasshouses it would have been manifested in at least some of the many series of observations carried out in this work. It is concluded therefore, that such stratification does not exist.

CARBON DIOXIDE IN RELATION TO GLASSHOUSE CROPS

PART II.

THE PREPARATION OF AN ATMOSPHERE RICH IN CARBON DIOXIDE

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(With 3 Text-figures.)

Before proceeding to elaborate experiments upon the fertilising effects of atmospheric carbon dioxide it is necessary to be able to prepare at will, atmospheres rich in this gas. A review of recent literature on the subject and more especially on the use of waste gases from furnaces shows that while carbon dioxide has been passed into experimental houses, no estimation has been carried out of the concentration in the vicinity of the plants. In the present investigations an attempt has been made to evolve a method of obtaining a given concentration which will persist.

Preliminary experiments in a normal cucumber house showed that the carbon dioxide disappeared as soon as it was put in. Part of this was doubtless taken up by the plants, but it is probable that the greater part diffused through the ventilators, glass overlaps and door. Soil absorption might also occur. To eliminate these factors in the present work the soil was covered with a layer of concrete, the overlaps were filled with putty, and the door and all the ventilators of a small chamber well padded. The results obtained under these conditions form the greater part of the present communication.

Even with these precautions it was found that the carbon dioxide disappeared almost as quickly as it was introduced when it was allowed to diffuse *slowly* into the house. Furthermore, the distribution was irregular. It was decided therefore to investigate the effect of mechanical mixing by means of a fan. By this means complete distribution was obtained, but there was still a serious initial loss of gas, only one half of the amount introduced being found on analysis of the atmosphere.

A similar loss was found when an attempt was made to introduce a large volume of carbon dioxide in a short time by the action of sulphuric acid on sodium bicarbonate. In addition much delay was continually caused by the frothing of the mixture and consequently it always required a considerable time to add the requisite amount of acid. Later, using purer acid, it was found that comparatively little frothing took place and the addition of acid took much less time. In these circumstances as much as 98 per cent. of the theoretical amount of gas was found in the chamber. These results indicate that rapid generation of the theoretical amount of carbon dioxide and mixing by mechanical means are essential in the preparation of an atmosphere containing a given concentration of carbon dioxide.

With the precautions described above, a concentration of 30 parts per 10,000 can be maintained, but this involves firstly, the production of the initial concentration by means of bicarbonate and secondly, a continuous supply of carbon dioxide and continuous mixing by means of a fan. This method is, at best, cumbersome, and consequently for the projected experiments an alternative one was sought. The most promising solution lay in the determination of the rate of disappearance of the gas. By this means it could safely be said that, providing the rate of loss was a regular one, the concentration over a given time must lie between two limits, the limits being determined by experimental conditions. At the end of a given interval enough carbon dioxide could be introduced into the chamber to bring the concentration up to its initial value and hence the concentration throughout the day could be kept at any required *average* value.

Actually it was found that the rate of loss was quite regular and further, the regularity persisted even when the fan was used for the initial mixing only. To give therefore an even distribution circulation is only necessary during the decomposition of the bicarbonate. The relative effects are well brought out in the curves in Fig. 5.

It had been suggested in the early stages of the investigation that soil absorption played an important part in the loss of carbon dioxide, and to test this a layer of soil was placed on the floor of the house without being dried or treated in any way. The curves showing average rate of loss with and without soil in the house agree in a very striking manner as shown in Fig. 5. That obtained with soil in the house lies slightly below the other, but it is possible that this is to be explained by the relatively high humidity in the presence of soil. The relative humidity of the air seldom fell below 70 per cent. and averaged over 80 per cent.

The presence of about 70 plants of various ages in the house was without material effect on the rate of loss of carbon dioxide.

Finally it is worthy of note that even with the high concentration prevailing after stopping the fan no indications of stratification were observed.

Experimental details. Timmis (3) has already described the early work on this subject. The same work was repeated and amplified by one of the present authors (see Part I). Briefly the results obtained were as follows.

The experimental chamber used was at the north-east corner of a partitioned glasshouse. Its capacity was approximately 1000 cu. ft., so that one cubic foot of carbon dioxide introduced would correspond to a concentration of ten parts per 10,000.

While cucumbers were growing in the house, carbon dioxide from a cylinder was introduced through a meter and a perforated lead pipe. No steps were taken to ensure mixing, and under these conditions Timmis found that when ten cubic feet of carbon dioxide had been added, the concentration one hour later was ten parts carbon dioxide one foot above ground and seven parts eight feet above. A hole two inches deep in the soil showed a concentration of 64 parts carbon dioxide. From these results Timmis suggests that the main cause of the loss of the gas is by absorption in the soil.

Experiments on circulation. Early in the present year a fan with suitable driving mechanism was erected. The arrangement of the inlet and outlet tubes and of the carbon dioxide supply and sampling tubes is shown in Fig. 3. In the first experiments the soil was covered with a six inch layer of concrete. To obviate the necessity of entering the house to take samples, glass tubes were passed through the corridor wall and projected three feet in the house. They were closed by rubber tubing and screw clips. Fig. 4 shows their relative positions. Samples were taken at the ends *A*. The analyses were performed with the portable Haldane Apparatus and for the higher concentrations with the Large Haldane Apparatus (2). The analyses were done in duplicate and each result given is the mean of two or three readings.

Carbon dioxide obtained from a cylinder. After preliminary trials it was found that between two and three cubic feet of gas could be passed into the chamber in an hour, a greater rate tending to choke the apparatus by freezing.

The carbon dioxide was at first conveyed into the inlet of the fan which was kept running while the gas was being introduced. About one-

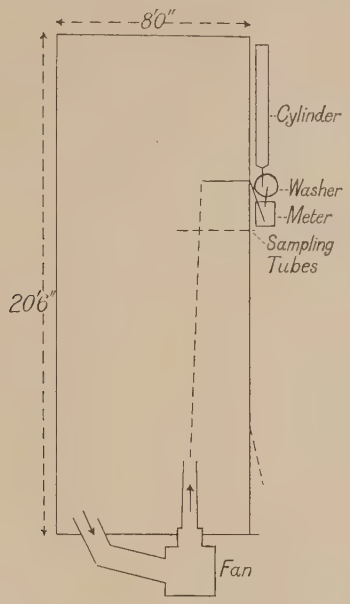

Fig. 3. Plan of chamber, showing fan, etc.

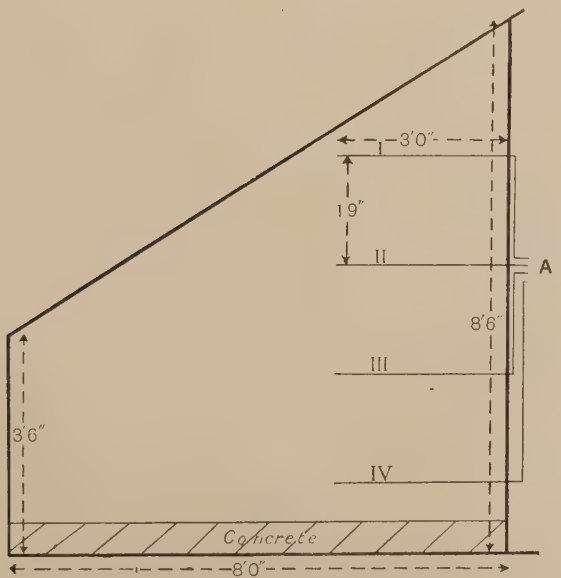

Fig. 4. Side elevation of chamber, showing sampling tubes (not to scale).

third of the carbon dioxide introduced could be registered and the distribution was complete. A set of typical results is given in Table V, the numerals referring to the sampling tubes shown in Fig. 3.

Table V. *Carbon dioxide from cylinder passed into inlet of fan.*

3·3 cu. ft. of carbon dioxide introduced should give 33·0 parts per 10,000.
Found:—15 minutes after gas was introduced.

I. 11·0 parts carbon dioxide
III. 10·7 „ „ „

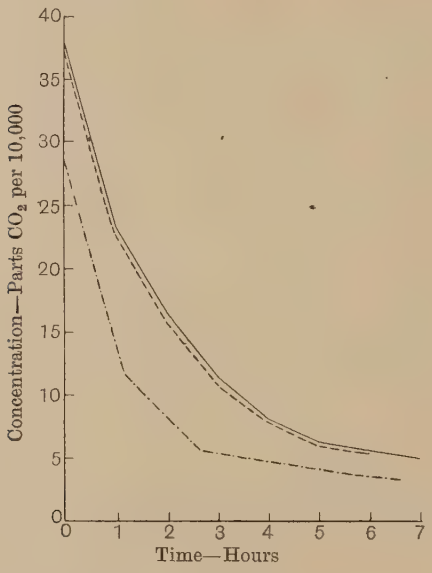

Fig. 5. Showing rate of loss of carbon dioxide:
(1) with continuous mixing ——•——•——•——
(2) mixing during introduction only—no soil ————————————
(3) mixing during introduction only—with soil — — — — — — —

The carbon dioxide was now allowed to drip in front of the outlet of the fan, so that it was immediately dispersed about the chamber. The results are shown in Table VI.

Table VI. *Carbon dioxide from cylinder conveyed to outlet of fan.*

Theoretically 25·0 parts per 10,000.
Samples taken immediately:
Found:

I. 14·2 parts carbon dioxide
II. 14·2 „ „ „
III. 13·9 „ „ „
IV. 13·7 „ „ „

Thus about half the carbon dioxide introduced could be found, and the distribution was complete.

These tables show the gas to be evenly distributed. Hence it is sufficient to take a sample through any one of the tubes as an indication of the average concentration at that time.

SODIUM BICARBONATE AS SOURCE OF CARBON DIOXIDE. To get an initial concentration higher than was possible by using a cylinder, sodium bicarbonate was decomposed by means of Rectified Oil of Vitriol. One and a half lbs. of bicarbonate were placed in a large pneumatic trough in the chamber and 30 per cent. acid was run in through sampling tube III until effervescence ceased. Theoretically 64·2 cu. ft. of the gas should be evolved from this weight of bicarbonate. Carbon dioxide was also passed in from the cylinder during the decomposition. About half the amount of carbon dioxide was found, *e.g.* 38·1 parts per 10,000 out of a theoretical 73·5 parts.

Using four and a half lbs. of bicarbonate, giving a concentration of 192–6 parts, 173 parts were found. The decrease in the initial loss is probably connected with the fact that three times the amount of bicarbonate was decomposed in only twice the time. With a purer acid the time was reduced still further and 193 parts were found. Similar results were obtained with smaller amounts of bicarbonate.

MAINTENANCE OF A GIVEN CONCENTRATION. The initial concentration was obtained by decomposing sodium bicarbonate, and carbon dioxide was then passed in from the cylinder. The supply of gas tends to fall off when it is allowed to evaporate rapidly owing to the cooling of the apparatus and hence the concentration gradually falls off.

Table VII. *Maintenance of a given concentration of carbon dioxide.*

Bicarbonate just decomposed	33·7	parts	carbon	dioxide
After 50 minutes	32·5	,,	,,	,,
,, 1 hour 35 minutes	30·3	,,	,,	,,
,, 2 hours 10 minutes	29·9	,,	,,	,,

The concentration rapidly fell off as soon as the fan and supply of gas were stopped. Thus after 45 minutes it had fallen to 13·6 parts per 10,000.

DETERMINATION OF THE RATE OF LOSS OF CARBON DIOXIDE. The first experiments were designed to discover if stopping the fan as soon as the evolution of gas had ceased would affect the rate of loss. In one case the fan was kept running all day and samples were taken every ninety minutes. The rate of loss approximated very closely to that found when the fan was stopped as soon as the bicarbonate was decomposed.

Table VIII. *Fan running all day.*

Theoretically 64·2 parts carbon dioxide per 10,000

Found:			
Bicarbonate just decomposed	28·5 parts	After 4 hours	4·7 parts
After 1 hour	11·7 „	„ 5½ „	3·6 „
„ 2½ hours	5·7 „	„ 6½ „	3·2 „

Table IX. *Fan stopped after decomposition of bicarbonate.*

Theoretically 73·5 parts carbon dioxide.

Found:			
Bicarbonate just decomposed	38·1 parts	After 4 hours	7·7 parts
After 1 hour	23·2 „	„ 5 „	6·1 „
„ 2 hours	15·4 „	„ 6 „	5·7 „
„ 3 „	10·4 „	„ 7 „	5·1 „

Effect of soil on rate of loss. Virgin soil from the field and not dried in any way was placed in the house in a layer about four inches deep. The addition of soil greatly increases the relative humidity of the air but this does not appear to affect the rate of loss.

Table X. *Soil in the chamber.*

Theoretically 71·5 parts carbon dioxide per 10,000.

Found:			
Bicarbonate just decomposed	37·1 parts	After 4 hours	7·1 parts
After 1 hour	21·6 „	„ 5 „	5·8 „
„ 2 hours	13·9 „	„ 6 „	5·8 „
„ 3 „	9·1 „		

Tables VIII, IX, and X are incorporated in Fig. 3.

Using four and a half lbs. of bicarbonate giving a concentration of 192·6 parts per 10,000, a similar type of curve was obtained although naturally it differed in slope.

Table XI.

Theoretically 192·6 parts carbon dioxide per 10,000.

Found:			
Bicarbonate just decomposed	168·8 parts	After 4 hours	36·3 parts
After 1 hour	110·9 „	„ 5 „	26·1 „
„ 2 hours	73·7 „	„ 6 „	18·7 „
„ 3 „	51·5 „	„ 7 „	13·3 „

With a purer acid 193 parts were found out of a theoretical 197. Samples taken during the decomposition of the bicarbonate showed that the gas was evolved at the rate of nearly one cubic foot per minute.

SUMMARY OF PARTS I AND II.

(1) As a preliminary to the examination of carbon dioxide as a fertiliser under glass the distribution of the gas in the glasshouses has been examined.

(2) Contrary to general belief it is shown that the gas is not distributed in layers. The concentration has been in certain cases artificially increased but even then no evidence of stratification is found.

(3) It is also shown that the average maximum concentration in the presence of cucumber plants occurs between 4 a.m. and 7 a.m. and the minimum between noon and 3 p.m.

(4) A method is described whereby atmospheres containing up to 200 parts per 10,000 of air can be prepared.

(5) The gas is prepared from sulphuric acid and sodium bicarbonate, and, providing an initial mixing takes place, it is uniformly distributed.

(6) It is shown that the distribution is independent of:

(*a*) the total amount of carbon dioxide present,

(*b*) the presence of soil,

(*c*) ,, ,, ,, plants.

(7) A definite loss of carbon dioxide always occurs, and a curve showing the rate of fall has been obtained.

Among the factors which may be responsible for this disappearance are leaks, soil, wood, glass, manure, plants and moisture. The present paper shows that soil and plants are without effect, and it is proposed at the end of the present growing season to examine the effects of the other factors and if possible to account for the high losses.

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CORRELATION BETWEEN LOSS OF LEAF AND DAMAGE TO CROP IN LATE ATTACKS ON WHEAT

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(With 6 Text-figures.)

The following experiment was undertaken to find a basis for estimating the effect on the yield of wheat by obtaining the percentage of loss of leaf surface.

Method.

Two varieties of wheat were chosen for the test, namely, Little Joss and New Red. As soon as the ears began to unfurl one yard lengths in the drills were treated in the following ways:

(*a*) all leaf blades were cut off,

(*b*) half of each leaf blade was cut off,

(*c*) all leaves on the bottom half of the plants were removed.

Fig. 1. Appearance of plants under test.

A series of three separate yard lengths of drills was obtained in this way on each variety.

The first plots were treated on July 2nd. A week later two other series, one on each variety, were similarly prepared. This was repeated each week until the crop was ripe and the leaves all withered. The last was prepared on August 15th making seven series in all. On the eighth week the crops were ripe and they were harvested on August 31st. In this way was obtained a succession of plots which had been badly damaged at regular intervals between flowering and ripening. The appearance of tillers under test is indicated in Fig. 1.

Compilation of Results.

There could obviously be no alteration in the number of ears produced because these were already formed when the test began. The effect of the damage could be on (*a*) size of ear, (*b*) size of grain, (*c*) a combination of these coupled with number of grains.

All the ears were collected on the yard drill lengths and the following measurements made: (1) average length of ear (average of ten on each yard length of drill), (2) average volume of 200 grains, (3) weight of grain on an average from ten ears.

For convenience the grains were rubbed out of ten ears and then weighed. No attempt was made to get in each case ten average ears, because all sound ears on the plot were weighed. The chief cause of the variation in the number of ears on the various yard drill lengths was damage by birds. All damaged ears were of necessity neglected.

For control and crop-average a number of groups of ten ears were obtained in different parts of the two fields and all averaged for the horizontal line of crop-average in each variety.

Data.

A study of the lengths of ten ears (*vide* Table I) gave no indication whatever of interference with the leaf area. The table shows clearly the greater length of the Little Joss ear than that of New Red, the average length of the former being 11 cm. compared with 9·16 cm. in the latter.

Table II gives the volume in cubic centimetres of 200 grains. It will be seen that the grain from the stripped plant was in general smaller than that from the half-stripped plants on adjacent drills. In only three cases were they equal and in no case did the grain from stripped plants develop better than that from the half-stripped plants. The grain from the bottom-stripped plants was very variable and the average ultimately

was the lowest. Although the figures are not sufficient to be of any use in determination of crop-loss it seems very clear that interference with the leaf area hindered considerably the filling out of the grain.

Table I. *Length of Ears.*

(10 ears in centimetres.)

Week	Stripped	Half-stripped	Bottom-stripped
Little Joss: Control 110·0			
1	108·5	96·1	103·3
2	—	—	—
3	104·4	119·2	101·5
4	104·8	101·2	103·2
5	92·7	109·9	106·5
6	105·4	107·1	102·1
7	105·5	107·8	104·3
New red: Control 91·6.			
1	88·7	78·6	82·8
2	93·1	85·6	83·8
3	87·0	85·6	86·8
4	87·1	82·2	89·5
5	89·9	91·0	89·4
6	92·4	94·3	96·4
7	91·8	—	—

Table II. *Volume of Grain.*

(c.c. per 200 grains.)

Weeks ...	1	2	3	4	5	6	7
New Red: Control 7·5 c.c.							
Stripped	6·0	5·5	7·0	6·5	5·5	8·0	7·0
Half-stripped	7·0	6·5	7·0	8·0	8·0	8·5	7·0
Bottom stripped...	7·0	7·25	7·25	7·5	5·0	5·5	7·0
Little Joss: Control 7·1 c.c.							
Stripped	8·0	—	7·0	6·5	8·0	8·0	8·0
Half-stripped	8·0	—	7·5	7·25	6·75	7·5	8·0
Bottom-stripped...	7·25	—	5·5	6·5	7·75	7·0	8·25

Averages:

	Number used	
Control	1000	7·3 c.c. per 200
Stripped	2600	7·0 „ „
Half-stripped	2600	7·46 „ „
Bottom-stripped	2600	6·85 „ „

The figures for the weighings of grain from successive groups of ten ears are given in Tables III and IV for the varieties New Red and Little Joss respectively. Where more than two sound ears were left after grouping in tens these were weighed and the figures are given in brackets with the number weighed indicated outside the bracket in each case.

Table III.

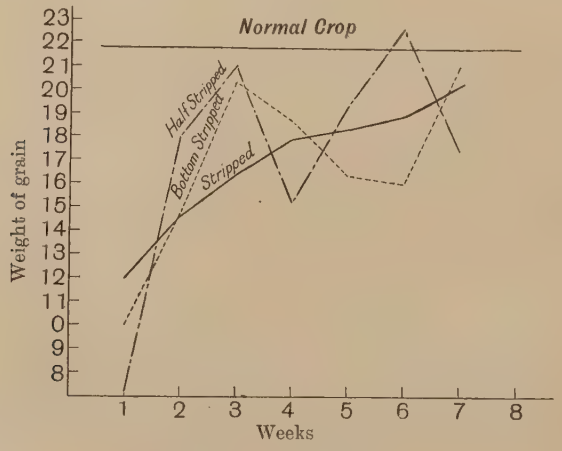
New Red	Weight of grain from 10 ears in grams (except where in brackets)	Average weight of grain in 10 ears	No. of ears weighed
Control	23·65, 19·34, 18·55, 23·31, 20·35, 22·75, 24·30	21·75	70
1st week:			
Stripped	11·07, 10·65, 14·23	11·98	30
Half-stripped	6·35, 8·10, 7·08	7·20	30
Bottom-stripped	8·01, 9·50, 11·73, (8·45)[8]	10·00	38
2nd week:			
Stripped	13·95, 15·92, 13·67, (10·38)[7]	14·57	37
Half-stripped	15·04, 17·35, 21·67	18·02	30
Bottom-stripped	13·36, 13·24, 13·09, 19·37, (5·35)[4]	14·64	44
3rd week:			
Stripped	14·78, 18·45, 14·95, 15·0, 17·02, 17·99	16·36	60
Half-stripped	21·24, 20·75	20·99	20
Bottom-stripped	22·32, 17·45, 17·91, 22·72, 21·27	20·33	50
4th week:			
Stripped	17·45, 15·57, 18·12, 20·58	17·93	40
Half-stripped	15·67, 15·75	15·71	20
Bottom-stripped	18·07, 14·79, 20·57, 23·68, (12·48)[8]	18·66	48
5th week:			
Stripped	20·15, 16·42	18·28	20
Half-stripped	19·54, 19·42, 20·63, 17·95, (7·39)[4]	19·30	44
Bottom-stripped	18·19, 14·96, (11·02)[7]	16·36	27
6th week:			
Stripped	17·60, 18·47, 20·76	18·94	30
Half-stripped	22·60, 22·77, 22·38	22·58	30
Bottom-stripped	18·37, 13·64	16·01	20
7th week:			
Stripped	18·30, 21·45, 22·75, 20·10, 18·85, 19·88	20·22	60
Half-stripped	17·45, 15·57, 17·50, 18·71, (5·41)[3]	17·36	43
Bottom-stripped	18·11, 22·20, 23·57, 21·34, 20·40, (9·77)[5]	20·98	55
			846

Study of these figures reveals a regular and close connection between the damage to the leaf and the weight of grain obtained. The loss of crop was large when the plants were younger and became steadily smaller as the plant approached maturity. This is further and better shown in Curves 1 and 2.

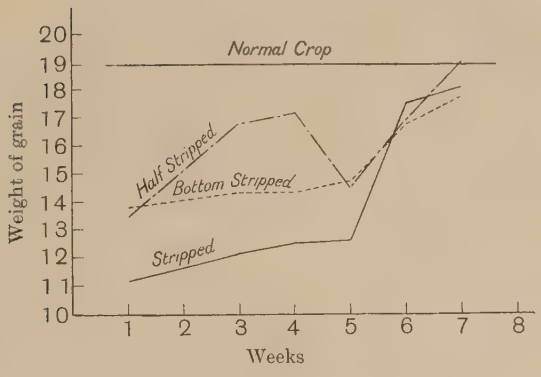
In Table V and Curve 3 the figures for the two varieties are combined to lessen varietal influence. These show more clearly the close connection between loss of crop and damage to leaf area. It seems clear that weight of grain from ten ears is a good unit for the purpose of this experiment combining as it does the relative plumpness of the grain with the number of grains produced.

Table IV.

Little Loss	Weight of grain from 10 ears in grams (except those in brackets)	Average weight of grain in 10 ears	No. of ears weighed
Control	20·99, 18·50, 16·32, 20·22, 19·47, 17·47, 18·47, 15·50, 22·45, 19·50	18·89	100
1st week:			
Stripped	11·91, 12·52, 9·46, 10·89	11·20	40
Half-stripped	14·04, 14·85, 16·60, 12·35, 9·22, 14·15	13·54	60
Bottom-stripped	12·07, 16·25, 12·11, 15·70, (6·40)[4], 11·95	13·79	54
2nd week:			
Stripped	(11·71)		
Half-stripped	(15·16)		
Bottom-stripped	(14·06)		
3rd week:			
Stripped	10·72, 15·10, 11·94, 11·92, (4·12)[4]	12·23	44
Half-stripped	16·81, 17·21, 18·54, 13·96, 18·60, (10·59)[7]	16·79	57
Bottom-stripped	15·84, 13·30, 13·89	14·34	30
4th week:			
Stripped	11·57, 12·65, 11·85, (7·8)[5]	12·53	35
Half-stripped	18·05, 17·10, 16·35	17·17	30
Bottom-stripped	16·16, 12·47	14·32	20
5th week:			
Stripped	12·45, 12·75	12·60	20
Half-stripped	14·95, 14·95, 12·56, 15·52	14·50	40
Bottom-stripped	17·61, 16·76, 13·47, 11·07, 14·90	14·75	50
6th week:			
Stripped	17·37, 18·17, 15·15	17·46	30
Half-stripped	17·92, 17·98, 14·44	16·78	30
Bottom-stripped	19·97, 20·45, 12·7, 11·3, 19·72	16·83	50
7th week:			
Stripped	20·94, 18·47, 19·17, 17·85, 17·01, 17·7, (14·20)[9]	18·16	69
Half-stripped	18·98, 21·17, 18·09, (8·15)[5]	18·97	35
Bottom-stripped	19·86, 15·26, 18·35, 17·62, (10·39)[6]	17·71	46
			840



Curve 1. Variety, New Red.



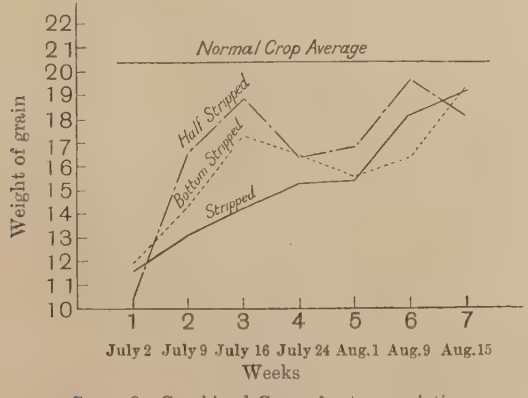
Curve 2. Variety, Little Joss.

Table V. *Figures for Little Joss and New Red combined.*

(Average weight of grain rubbed from 10 ears, in grams.)

Control: Little Joss 18·89; New Red 21·75; Combined 20·32.

Weeks ...	1	2	3	4	5	6	7
Stripped:							
Little Joss	11·20	11·71	12·23	12·53	12·60	17·46	18·16
New Red	11·98	14·57	16·36	17·93	18·28	18·94	20·22
Combined	11·59	13·14	14·29	15·23	15·44	18·20	19·19
Half-stripped:							
Little Joss	13·54	15·16	16·79	17·17	14·50	16·78	18·97
New Red	7·20	18·02	20·99	15·71	19·30	22·58	17·36
Combined	10·37	16·59	18·89	16·44	16·90	19·68	18·16
Bottom-stripped:							
Little Joss	13·79	14·06	14·34	14·32	14·75	16·83	17·71
New Red	10·00	14·64	20·33	18·66	16·36	16·01	20·98
Combined	11·89	14·35	17·33	16·49	15·55	16·42	19·34



Curve 3. Combined Curve for two varieties.

General Notes and Deductions.

The crops were rather late in flowering and fertilisation took place during the first week of July. On the 8th week, August 22nd, the leaves had all withered so no plots were made. Harvest took place on August 31st, the produce on the plots being taken indoors at once.

Interference with the leaves on such a scale as in this experiment produced a very serious alteration of crops during the period of fertilisation, approximately reducing it 50 per cent. in all three cases and in both varieties. This great depreciation of the crop and crossing of the curves seems to suggest a physiological disturbance. It seems undesirable to include the period of fertilisation in the rectification of the curves until it has been further studied and for this reason it is omitted in such rectification given below.

The bottom stripped plants consistently cropped low, suggesting that the lower leaves play a considerable part in grain formation. These leaves are larger than the upper ones so that the crop would be between the Stripped and Half-stripped plants assuming all leaves to have equal value for grain formation.

The Control crop averages bear out the superiority of New Red over Little Joss for the district (Shropshire). This is in accordance with several years' trials.

The greater irregularity of the New Red curve is partly due to its being a variety specially suited to the locality and therefore better able to take advantage of soil, manures, etc.

The drop in the curve for Little Joss in the 5th week was due to an attack of Yellow Rust. As this was on the lower leaves it did not appreciably affect the Stripped or Bottom-stripped plants.

There is a general upward trend in the curves from the 2nd to the 7th week and a study of the average figures for the crop depressions shows a very complete correlation between leaf blade loss and diminished crop. This seems to justify rectification of the curves for statistical purposes in recording crop loss by measurement of leaf loss.

Straightened Curves for Stripped and Half-Stripped Plants.

Curve 4 gives the mean straight curve prepared from Curve 3.

Line *A* is the curve for the Stripped plant. This shows the gradual diminution of the crop depression from the yield 12·76 in the 2nd week to reaching full crop 20·32 of the untouched plant at 8th week.

Line *B* is obtained by halving the crop alterations to correspond to half-stripping.

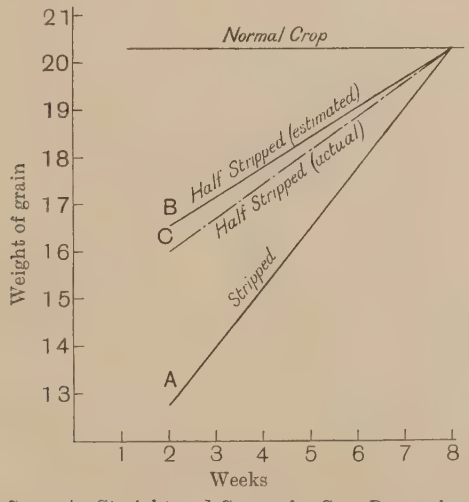
Line *C* is the straightened curve from the results actually obtained in the field and corresponds very closely to line *B* obtained as above indicated.

From these Curves *A* and *B* the total crop depression at the various weekly intervals can be stated, thus:

Table VI.

	Percentage crop obtained	
	Stripped	Half-stripped
2nd week	62·8	81·4
3rd „	69·0	84·5
4th „	75·2	87·6
5th „	81·4	90·7
6th „	87·6	93·8
7th „	93·8	96·9
8th „	100·0	100·0

This provides a basis for calculating the effect on the cereal yield of various leaf-attacking pests.



Curve 4. Straightened Curves for Crop Depression.

Percentage Alteration of Crop during the test.

A. During Fertilisation (1st week):

Normal Crop $3 \times (\text{average}) = 3 \times 20{\cdot}32 = 60{\cdot}96$

Depressed crop $\{(\text{Stripped}) 11{\cdot}59\} + \{(\text{Half-stripped}) 10{\cdot}37\} + \{(\text{Bottom-stripped}) 11{\cdot}89\} = 33{\cdot}85$

i.e. Normal Crop ... ... 100 %

Crop obtained ... ... 55·5 %

B. Average depression during the next six weeks:

Normal crop $6 \times 20{\cdot}32 = 121{\cdot}92$

Crop from stripped plants:

$= 13{\cdot}14 + 14{\cdot}29 + 15{\cdot}23 + 15{\cdot}44 + 18{\cdot}20 + 19{\cdot}19 = 95{\cdot}49$

Crop from half-stripped plants:

$= 16{\cdot}59 + 18{\cdot}89 + 16{\cdot}44 + 16{\cdot}90 + 19{\cdot}68 + 18{\cdot}16 = 106{\cdot}66$

Crop from bottom-stripped plants:

$= 14{\cdot}35 + 17{\cdot}33 + 16{\cdot}49 + 15{\cdot}55 + 16{\cdot}42 + 19{\cdot}34 = 99{\cdot}48$

i.e.

Normal crop	100 %
Stripped crop	78·3 %
Half-stripped crop ...	87·5 %
Bottom-stripped crop ...	81·6 %

These figures further illustrate the regularity of crop depression due to loss of leaf surface. They are represented in diagrammatic form in Fig. 2.

Fig. 2.

Use of Table VI or Curve 4.

An attack of *Agromyza ambigua* was observed on Marshal Foch wheat during 1922. Area 19 acres. Yield 48 bushels per acre. The attack was around 3rd to 7th weeks.

Percentage of attacked plants ...	10
Leaf surface of plant lost ...	·2
Leaf surface of field lost ...	2 %
Depression for total loss of leaf $100 - 81{\cdot}4 =$	18·6 %
Depression for 2% loss of leaf $\frac{18{\cdot}6}{50} =$	·37 %
Yield obtained = 912 bushels per 19 acres =	99·63 % full crop
Full crop should have been ...	915·5 bushels,
or the loss due to this pest was ...	3·5 ,,

This is used in illustration purely. Such great accuracy is never likely to be obtained or required but it serves to classify this pest as one of the lesser evils of the wheat crop although it looked much worse in the field.

(*Received January 10th*, 1923.)

THE KILLING OF *BOTRYTIS CINEREA* BY HEAT, WITH A NOTE ON THE DETERMINATION OF TEMPERATURE COEFFICIENTS

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(With 5 Text-figures.)

In a previous paper(1) some results were given of a study of the action of phenol on the spores of *Botrytis cinerea*. The present communication contains the results of a similar enquiry into the action of moist heat. The method employed was essentially the same as that used in the phenol experiments, and consists in dropping spores of the organism, grown always under the same conditions and for approximately the same time, into distilled water of the temperature under investigation, removing samples from time to time, and determining for each sample the proportion of spores able to germinate on Czapek's agar at a temperature of 24–25° C. The strain used was the same as that previously described.

In determining the proportion of germinable spores a difficulty was experienced which was not met in the phenol work. In the latter case little or no delay was found in the germination of spores capable of development, practically all the viable spores germinating within 24 hours. This however is not the case after exposure to heat. While most of the germinable spores do put out their germ-tubes within 24 hours of transference to the nutrient medium, there is regularly a significant proportion which delays doing so for a period that is longer or shorter according to the temperature to which the spores have been exposed and the duration of the exposure. For example, after an exposure of three hours to 37° C. a sample was transferred to Czapek's agar and counted after 24 hours' incubation at 24–25° C.: out of 710 spores 49·8 per cent. were found to have germinated. After a further 24 hours' incubation the same slides recounted showed 74·43 per cent. of the spores to have germinated; and after 24 hours more the number was found to be 68·2 per cent. The fall in the last count is experimental and illustrates the difficulty of

accurate observation in a preparation which after 72 hours' incubation has become greatly overgrown. The hyphae from the spores which germinate early form appressoria and cover over many spores, so that at the last count, for example, only 324 spores could be counted out of the 710 known to be present in the preparations. Again, after 6 hours' exposure to a temperature of 37° C. the numbers germinated after a further 24 hours at 24°–25° were 2·3 per cent. out of 1300 present; 3·9 per cent. after 48 hours and 6·6 per cent. after 72 hours, after which time no further increase took place. With short exposures, on the other hand, *e.g.* $1\frac{1}{2}$ hours at 37°, the number germinated in 24 hours does not increase on further incubation. This delay indicates that the

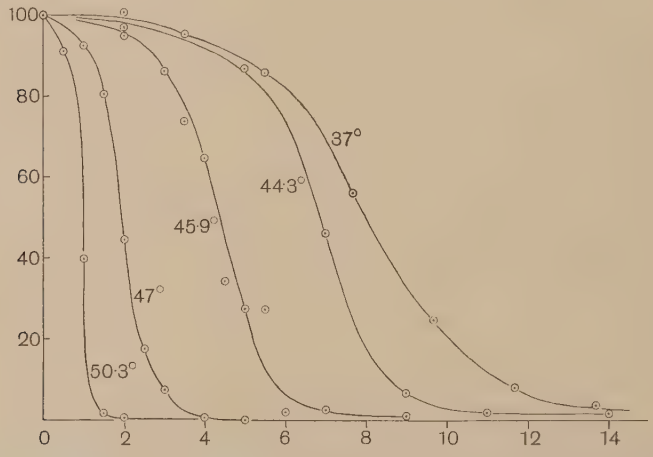

Fig. 1. Percentage of spores surviving plotted against time (abscissae). The time-scale represents minutes for the four higher temperatures; and half-hours for the 37° curve.

killing is a gradual process, and that the spore may be considerably damaged, may be in fact "half-dead," and yet recover sufficiently after a time to resume its normal course of development. It was not determined whether these late-developing spores showed other signs of altered vitality but no such alteration was observed. Weiss (2) has noted a similar effect of heat on the spores of *Bacillus botulinus*—the longer the period of heating before death occurs, the longer the period necessary for the subsequent germination of the spores.

The results obtained are shown in Tables I—V, and the data are charted in Fig 1. In this graph the abscissae represent time from the start of the experiments, the ordinates the percentage of survivors. The time scale for the four higher temperatures is in minutes but for the

results at a temperature of 37° C. it represents half-hours. It will be seen that the process of killing falls into two stages, a stage during which progressively increasing numbers of spores are killed in successive units of time, and a stage in which progressively diminishing numbers are killed. The curve is sigmoid in shape, with the point of inflection at the time when the maximum number of spores is being killed, and the two halves of each curve are approximately symmetrical.

Table I.

Temp. 37° C. 400,000 spores in 1 c.c. Control: 87·09 % germinated.

Sample taken in *mins.*	Number counted	Percent. germinated	Percent. calculated*
0	984	100·0	100·0
60	1150	100·4	98·0
106	1217	94·62	94·5
182	710	85·46	86·6
230	814	55·86	56·3
290	1076	24·41	24·5
352	1657	7·96	7·9
410	987	3·6	2·7
1380	1500	0·0	0·0

* See p. 341.

Table II.

Temp. 44·3° C. 440,000 spores in 1 c.c. Control: 92·57 % germinated.

Sample taken in *mins.*	Number counted	Percent. germinated
0	1750	100·0
2	1314	96·2
5	1249	86·5
7	695	45·8
9	896	6·38
11	997	1·72
14	1261	1·44
20	1267	0·24
40	1600	0·129

Table III.

Temp. 45·9° C. 494,000 spores in 1 c.c. Control: 95·30 % germinated.

Sample taken in *secs.*	Number counted	Percent. germinated
0	897	100·0
120	770	95·3
180	650	86·0
210	782	73·4
240	1003	64·5
270	1163	34·0
300	654	27·3
330	1262	27·02
360	1523	1·99
420	1659	2·46
450	1603	0·85

Table IV.

Temp. 47° C. 570,000 spores in 1 c.c. Control: 92·74 % germinated.

Sample taken in *secs.*	Number counted	Percent. germinated
0	964	100·0
62	1237	92·3
92	1179	80·3
122	1396	44·4
152	1099	17·5
182	1761	7·22
242	1267	0·603
302	1214	0·0

Table V.

Temp. 50·3° C. 930,000 spores in 1 c.c. Control: 97·24 % germinated.

Sample taken in *secs.*	Number counted	Percent. germinated
0	1595	100·0
30	1757	91·0
60	1528	39·8
90	1839	1·33
120	1806	0·34
240	1834	0·17
300	1900	0·05
360	1900	0·0

The series of curves bears a general resemblance to that obtained with phenol used in increasing strengths (1), but there is a difference in the two sets which points to a difference in the mode of action of the two killing agents. In the case of phenol the shape of the curve gradually changes as the strength of the poison is increased, the sigmoid character tending gradually more and more to disappear owing to the flattening out of the first elbow or bend in the curve. Eventually with a sufficiently high dose of phenol the sigmoid shape is completely lost and is replaced by a logarithmic type of curve, corresponding to that of a unimolecular chemical reaction. With heat as the killing agent there is no tendency for the curve to change into the logarithmic type. The curve remains the same throughout, and the process runs alike at all the temperatures, the only difference being in the rate at which it occurs.

This may be seen by comparing the times required to kill 25 per cent., 50 per cent. and 75 per cent. of the spores at the different temperatures with the times required at 50·3° C.

Table VI.

	Time in mins. required to kill			Ratio of the time at each temp. to the time at 50·3°		
	25 %	50 %	75 %	25 %	50 %	75 %
31°	9240·0	12140·0	—	11922·0	13202·0	—
37°	199·0	240·0	286·0	256·0	261·0	263·0
44·3°	5·8	6·8	7·84	7·4	7·4	7·2
45·9°	3·56	4·32	5·0	4·5	4·7	4·5
47°	1·65	2·0	2·33	2·1	2·2	2·1
50·3°	0·775	0·918	1·087	1·0	1·0	1·0

As Table VI shows, the time ratios at each temperature remain practically the same for all the stages of the process, which would not be the case if there were any significant change in the shape of the curves.

The same fact is perhaps even more clearly shown in Fig. 2, where all the observations at all the temperatures are plotted on the one diagram. The curve is drawn directly from the formula of Student's curve (3), discussed later, the time to kill 50 per cent. of the spores being taken as 1 on the abscissa line, and the ordinates representing the proportion of spores killed. For each of the temperatures studied, the time found to be necessary to kill 50 per cent. of the spores is similarly taken as 1, and the observed results entered on the figure correspondingly. For example, at 37° C. the time required to kill 50 per cent. of the spores is 240 minutes, which is taken = 1 on the time scale. The observed time of 106 minutes therefore corresponds to 106/240 = ·442 on the figure,

and at this time 94·62 per cent. spores were found surviving, *i.e.* 5·38 per cent. had been killed. It is evident that all the observations at all the temperatures are arranged closely along the curve, although at 37° C. the process requires over eight hours for completion and at 50·3° C. only three to four minutes. The relative susceptibility of the individual spores remains the same in spite of the very great increase in activity of the killing agent.

As we have seen, this was not the case with phenol. A spore which was five times as resistant as another to a low strength of phenol was not five but more than five times as resistant to a high strength. In the previous paper this change was attributed to an alteration in the

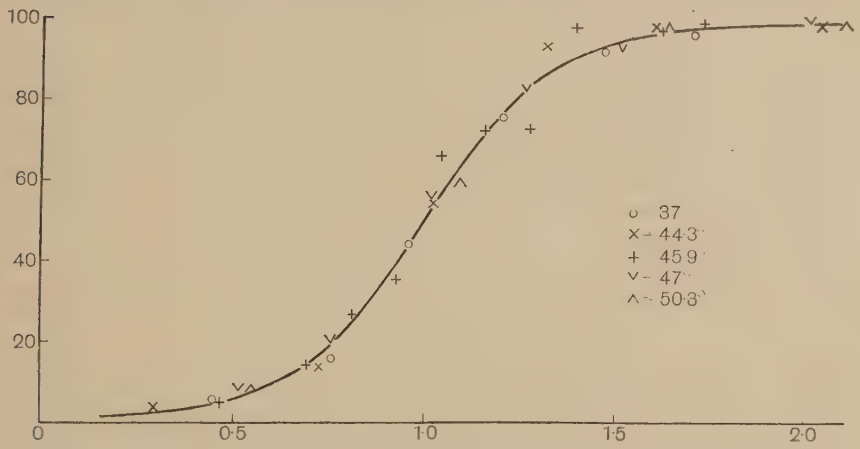

Fig. 2. The observations at five temperatures plotted on the one chart. The curve is drawn from Table XXV in *Tables for Statisticians and Biometricians*, with $n = 5$. The abscissae represent times, the time required to kill 50 % of the spores being taken as 1 for each temperature. The ordinates represent percentage of spores killed.

relative permeability of the individual spores as the poison was increased in strength. There are, we may suppose, two stages necessary in the process of killing of any spore by phenol. There is, first, the stage of penetration, during which the poison is making its way through the external coat or wall of the spore and, secondly, the stage of coagulant or other action on the spore contents after penetration has occurred. It was to a change in the relative rapidity with which the first stage was effected that the change in curve-shape was attributed; and this view is supported by the absence of the change in the heat experiments, because with heat the first stage is virtually eliminated. The *Botrytis* spore, a small body measuring $12\mu - 16\mu$ in its longest axis, takes, with only

a slight delay, the temperature of the water in which it is suspended and the difference in time between the death of two spores of the same suspension (a difference, it may be, of hours) can scarcely be due to any significant extent to a delay in the heating of the spore which survives the longer. The differences in the times of death are due to differences in resistance of the spore-contents to the action of heat. This action which, as we have seen, proceeds gradually, may take long periods to reach completion and allows of the occurrence of the delayed germination already described.

The Shape of the Curve. The shape of the curve at once suggests a frequency distribution of resistances to killing among the individual spores, and this seems the most reasonable of the explanations that might be offered. Inspection suggested that the distribution would be nearly normal, and this apparent normality was tested by expressing each time interval as a fraction of the time taken to kill 50 per cent., and plotting

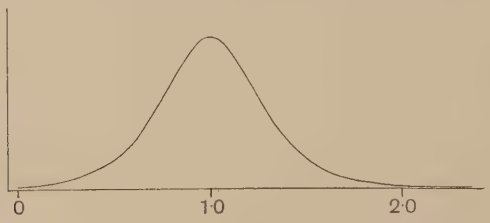

Fig. 3. Curve showing the distribution of spore-resistances.

these as abscissae against the percentages killed, expressed as deviates of the normal curve. The results of all five temperatures were plotted on one diagram; and if the proportions dying at the various times were normally distributed, these points would approximate to a straight line. There was however a distinct bending away in opposite directions at either end of the range, indicating that the distribution is not fully described by the normal curve but would be better represented by a curve such as that investigated by Student (3). Using Table XXV, given in *Tables for Statisticians and Biometricians* (4), with $n = 5$, and plotting the values of z against the time scale as before, the points were found to be closely grouped along a straight line. There was no tendency to an S-shape although a slight scatter occurred at the longer exposures (possibly due to post-mortem leaking of the contents of the spores first killed). At the five temperatures examined, therefore, the distribution of susceptibility to death at successive intervals of time is well described by Student's curve, of which an illustration is given in Fig. 3 to show the

type of distribution. This curve was used in drawing the curve in Fig. 2, where it will be seen that it fits the observations at all the temperatures very well indeed, especially when we consider that the experiments charted were carried out over a period of some months. With it we can represent with considerable accuracy the course of the reaction at any temperature within the limits examined, if the time required to kill 50 per cent. of the spores is known. In the last column of Table I are set out alongside the observed values the calculated values to be expected at the times indicated and it will be seen that the agreement is very satisfactory.

Temperature Coefficients. From Table VI (*supra*, p. 338), in which are given the times required to kill 50 per cent. of the spores at temperatures ranging from 31° C. to 50·3° C., it is evident that the influence of temperature on the rate of the reaction is remarkably great. At 31° C. the time is 12,140 minutes, at 50·3° C. it is only 0·92 minute, *i.e.* a rise of temperature of 19° C. has accelerated the reaction 13,200 times. The rise from 37° C. to 47° C. increased the velocity 120 times. Further, the temperature coefficient varies rapidly throughout the range of the temperatures examined. In Table VII are set out the coefficients for 1° C. rise (Q 1) over the intervals named, and the corresponding values for 10° C. (Q 10), if the same rate of increase had been maintained.

Table VII.

	Q 1	Q 10
31° –37°	1·92	690·0
37° –44·3°	1·63	132·0
37° –47°	1·61	120·0
44·3°–47°	1·57	92·8
47° –50·3°	1·40	29·5

In most chemical reactions the effect of a rise of 10° C. is to increase the velocity two or three times, so these coefficients in the case of *Botrytis* and moist heat are enormously high. There are however instances in the literature where similarly high coefficients have been obtained with similar material and heat; *e.g.* with agglutinins (5), bacterial toxins and other antigens (6) a coefficient of 2 for 1° C. rise (1024 for 10° C.); crystalline egg-albumen (7), 1·91 for 1° C. (646 for 10° C.); *Bacillus typhosus* (8), 1·63 for 1° C. (136 for 10° C.); *staphylococcus* (8), 1·4 for 1° C. (29 for 10° C.); *crystalline haemoglobin* (7), 1·3 for 1° C. (13·8 for 10° C.); *Bacillus coli* (8), 1·28 for 1° C. (12 for 10° C.).

It is perhaps scarcely to be expected that the relationship between temperature and velocity of the reaction found in these experiments

should be adequately expressed by the formulae of van t' Hoff or of Arrhenius. These formulae are empirical and were devised to meet the case of chemical reactions in which the velocity changes slowly over a long range of temperature, and not such a case as the present where the velocity is changing very greatly over a short range. It is however a remarkable fact that the formula of Arrhenius does express the relationship with great exactness, if we reckon the temperature, not from the absolute temperature of $-273°$, as Arrhenius does, but from 0° C. The values of the expression $\frac{T_0 T_n}{T_0 - T_n} \log \frac{t_n}{t_0}$ are given in the following table, in which the temperature T_0 is taken as 37° C. and the time t_0 as 240, the 37° C. experiment being probably the best observed of the series.

Table VIII.

	$\frac{T_0 T_n}{T_0 - T_n} \log \frac{t_n}{t_0}$
31°	325·6
37°	—
44·3°	347·5
45·9°	334·8
47°	361·5
50·3°	338·3
Mean:	341·5

The results show a very good approach to constancy. Further, if

$$\frac{T_0 T_n}{T_0 - T_n} \log \frac{t_n}{t_0} = k, \text{ then } \log t_n = \frac{K}{T_n} - \left(\frac{K}{T_0} - \log t_0\right) = \frac{K}{T_n} - A,$$

a constant, since t_0 and T_0 are known and constant; and the values of $\log t_n$, plotted against $\frac{K}{T_n}$, or the reciprocal of T_n, should lie on a straight line. This has been done in Fig. 4, and it will be seen that the observations at all the temperatures do fall upon a straight line.

This expression, then, enables us to deduce the time that would be required to kill 50 per cent. of the spores at any temperature within the range of 31° C.-50° C.; and, by the aid of the other formula for the curve already given, the whole curve of the process of the killing can be drawn for any temperature within these limits. The whole process is, in fact, expressed by these two formulae for the spores of *Botrytis cinerea*, of the strain and under the conditions used in these experiments.

It is of interest to consider what is to be expected at temperatures below 31° C., *i.e.* on prolonging the line of Fig. 4 upwards. At 31° C. the temperature of the last observation on the figure, one is on the confines

of the zone where death ceases and growth occurs. In distilled water at this temperature the organism dies: in a good nutrient medium it germinates and grows sparingly for a time. At 25° C. it grows well in a nutrient medium, and even in distilled water it germinates and grows freely for a time if it can obtain sufficient oxygen. At 25° C. in distilled water it dies if the oxygen supply is insufficient. If the spores are sunk in water to a depth of six inches, they do not receive enough oxygen for their development, and under these conditions they do not simply remain alive and inactive but die very slowly. After 37 days at this temperature 23·5 per cent. of the spores are already dead—a figure which, though greater than the expectation, is not so far removed from it but that the death may reasonably be attributed in large measure to the action of the temperature. Death from heat occurs even at temperatures very suitable for growth if a necessary condition of the growth be withheld. In fact one might say that all the spores would eventually be killed even at 25° C. and probably lower temperatures by the heat, if it were not for the occurrence of the opposing process of growth. Further consideration of this important question, however, must be postponed.

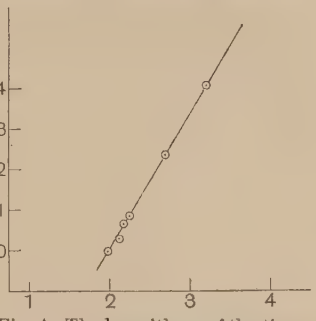

Fig. 4. The logarithms of the times taken to kill 50 % of the spores plotted against the reciprocals of the corresponding temperatures (abscissae).

Note on the Determination of Temperature Coefficients. In biological studies a usual way of estimating the effect of temperature upon a process is something as follows. When growth—to take this as an example—is studied, parallel cultures—whether of plants, fungi, bacteria or whatever is the subject under investigation—are set up usually in series at the different temperatures to be examined, and after the lapse of a certain time (an hour, a day, a week or whatever it may be) the amount of growth that has occurred is measured. If the result of such determination shows that twice as much growth has occurred at one temperature as at another, then it is said that a rise of so many degrees has doubled the rate of growth. This statement, of course, is true enough if one defines the word rate appropriately; and it would be true in general, if the growth or other process proceeded at a uniform speed throughout the life of the growing organism, or at least throughout the period of observation. But if that be not the case, and only rarely is it the case, to deduce the effect of temperature by such a method is misleading,

because it is applicable only to the special time selected. If, instead of taking one day or hour, the observations were made after two days or hours, a different result would be obtained; and so after three, four or any other number of periods: each will give a different result. In short, the result obtained depends on the time arbitrarily taken as the period of observation.

An illustration from a chemical reaction will make this clear. In Fig. 5 are plotted the amounts of work done in successive units of time, when the reaction is going at two different rates, one twice that of the other—*i.e.* corresponding to a temperature difference of about 10° C. It is apparent that if one compares the amounts of work done after equal periods of time, the result will vary according to the period selected. A short time will give nearly twice as much work done by the quicker

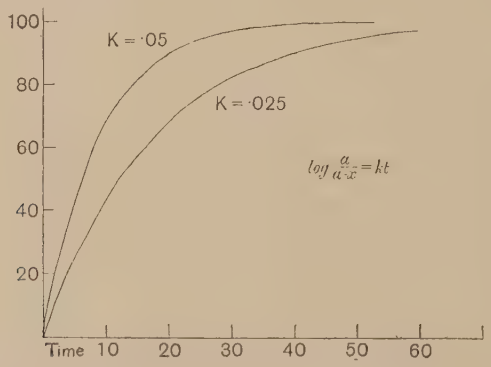

Fig. 5. Curve of unimolecular reaction proceeding at two rates, one twice that of the other.

reaction, a longer time only one and a half times as much, whilst a still longer time will give almost the same amount done by both. If these were reactions running at different temperatures, one would say that the effect of the rise of temperature had been to double the rate, or to raise it only one and a half times, or even to make practically no difference, according to the time one allowed to elapse before making the comparison. One would not get a true answer at any finite time.

There are two ways of looking at *rate*. One may take it as the amount of work done in a fixed time; which is the way we have been considering, or one may take it as the time required to do a certain amount of work. If the process under examination is going at uniform speed throughout, the two methods will give the same answer. The lines in the figure would be straight lines at different angles. But if it is not going at uniform speed, it is not indifferent which way is chosen, because different results are

obtained. In the figure, we have seen that the first way gives variable results; but the second way gives a constant and correct result. The time required to do the same amount of work is always double what it is in the other, whatever the amount of work we select as the standard. It would seem that this must be the correct method to employ in order to arrive at a true estimate of the effect of temperature[1].

An actual example from a recent most interesting and careful study by Fawcett (9) of the growth in diameter of fungal colonies will serve to illustrate the discrepancies found in practice. Fawcett found the temperature coefficient of growth of Pythiacystis for 10° C. rises to vary according to the time of observation over a long range of temperature, from 8° C. to 31° C., and tabulates the results accordingly. Two examples, taken from his paper, are given in Table IX. If however from his data one determines the times required for the colonies to reach a certain size and calculates the coefficients from these figures, one obtains the results shown in the second half of the table, viz. consistent values. The method of calculating from the results reached in constant time gives values which vary with the time chosen; whereas the other method of calculating from the times taken to reach a constant result gives values which are consistent and independent of the standard chosen.

Table IX.

Temp. Range	Coeff. calculated from amount of growth in			Coeff. calculated from time required for a growth of	
	1st day	2nd day	4th day	12·5 mm.	25 mm.
12°–22°	4·4	3·2	2·9	2·58	2·65
18°–28°	2·2	1·5	1·2	1·60	1·50

Again, in Lehenbauer's frequently quoted paper on the growth of maize seedlings (10), the temperature coefficients are calculated from the sizes reached after 12 hours' growth, at different temperatures from 12° C.–43° C.; and, as the writer himself points out, are valid only for the time period selected. From the data given the times required to reach definite limits of size can be ascertained, and from these times the coefficients have been calculated for the sizes of 1 mm., 4·8 mm. and 7 mm. (the data do not cover greater sizes except for a few temperatures). The results differ considerably from the coefficients obtained by Lehen-

[1] While this paper was in the press, it was found that this method had been pointed out and discussed by Osterhout in 1918 (11). Cf. also Osterhout's monograph (12).

bauer's method, but are reasonably consistent with one another as may be seen from the examples given in Table X.

It is not, of course, to be expected that even by the second method consistent results shall be obtained throughout the whole range of temperatures and the whole range of growth. This could only happen if the growth at all temperatures proceeded in similar fashion but at different speeds; and, as one knows, this is not the case: the shape of the growth curve alters markedly at high temperatures, especially above the optimum. This is a fact which affects all methods of calculating coefficients in such processes as growth. Even so, however, the influence of temperature on the rate of the process is more accurately represented by the second method than by that commonly adopted in biological literature.

Table X.

Temp. range	Coeff. calculated from growth reached in 12 hrs.	Coeff. calculated from times required to grow		
		1 mm.	4·8 mm.	7 mm.
14°–24°	4·17	3·1	3·1	3·1
23°–33°	1·57	1·36	1·42	1·45

I have pleasure in thanking Mr R. A. Fisher and the Statistical department at Rothamsted for assistance and advice in the application of the frequency curve used in this paper.

Summary.

The spores of *Botrytis cinerea* were exposed to the action of hot water at temperatures ranging from 31° C. to 50·3° C., the proportion of spores surviving at different times determined, and the results plotted for each temperature. These give a series of approximately symmetrical sigmoid curves, all exactly alike one another except for the change in the speed of killing at different temperatures. The curves are superimposable on adjustment of the time scale, and in this respect they differ from the curves obtained with phenol, where the shape changes from an S to a J shape as the strength of phenol is increased, eventually becoming logarithmic.

The shape of the general curve agrees excellently with a recognised type of frequency distribution, and the observations at all the temperatures examined fall closely on the curve of this distribution.

The effect of temperature on the velocity of the reaction is unusually great, and is well expressed by the formula of Arrhenius, if the temperature is reckoned from 0° C. instead of the absolute temperature.

By combination of the formula for the curve and the formula for the velocity-temperature relationship it is possible to express completely for the spores of *Botrytis* the whole of the killing process for any temperature within the limits studied under the conditions and with the strain used in these experiments.

It is pointed out that in the estimation of temperature coefficients comparison of the times taken to reach a constant result give more accurate and consistent values than comparison of the results reached in constant time.

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OBSERVATIONS ON THE BIONOMICS OF THE APPLE-BLOSSOM WEEVIL, *ANTHONOMUS POMORUM*, LINN.[1]

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(*From the Agricultural and Horticultural Research Station, Long Ashton, Bristol.*)

(With Plates XIX and XX and 11 Text-figures.)

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1. INTRODUCTION.

Year by year much damage is caused by the larvae of *Anthonomus pomorum*, Linn., the Apple-Blossom Weevil, and enquiries as to preventive and remedial measures are constantly received at the Advisory Centres.

[1] A grant in aid of publication has been received for this communication.

In view of this, and the fact that the life history and habits are, apparently, assumed rather than clearly understood, it was thought advisable to spend a considerable time on the careful observation and recording of the facts of the life history, habits and limiting environmental factors, if any, of this weevil.

Thus after some discussion of the matter with Mr A. Roebuck, Adviser in Entomology, Harper-Adams Agricultural College, and Mr A. H. Lees, M.A., Entomologist and Plant Pathologist at Long Ashton, it was decided to undertake the work. Accordingly, in September 1920, an excursion into the Pershore district of Worcestershire was arranged and the weevils hunted down in some of their winter quarters and a supply secured for laboratory work during the winter.

The laboratory work, through the kindness of Professor B. T. P. Barker, M.A., was carried out at the Agricultural and Horticultural Research Station, Long Ashton, under the supervision of Mr A. H. Lees whose constant advice and suggestions have been invaluable; the outside observations have been made, for the most part, in the plantations of the Research Station supplemented by those collected in Worcestershire and in Gloucestershire.

2. METHODS AND MATERIAL.

The scheme of work was arranged as follows:

September, 1920. Inspection of plantations to discover traces of, or winter quarters of, the weevil.

October, 1920 *to March*, 1921. Weevils kept under observation in the laboratory where numerous specimens were dissected with a view to becoming conversant with their anatomy and physiology and to distinguish the sexes. At the same time preliminary spraying trials were conducted from which the most promising were selected for plantation trials. By keeping the adults indoors in the warmer conditions and by close observation of their behaviour in spring, it was possible to follow, and to anticipate, to some extent, their activities in the plantations.

March, 1921 *to May*, 1921. Outside observation of habits. Indoor control of life history.

May, 1921 *to June*, 1921. Observation in plantations of habits of young adults.

July, 1921 *to September*, 1921. Observation in plantations of feeding habits and choice of winter quarters.

September, 1921 *to Spring*, 1922. Spraying trials[1] at Long Ashton and in Worcestershire.

Spring, 1922 *to early Summer*, 1922. Verification of habits and life history and collection of information as to the efficiency of the sprays used.

3. LOCALITY AND DISTRIBUTION.

Since local conditions vary to such a great extent and with them, more or less, the habits of any particular insect, it will perhaps be helpful to say a few words about this particular district in connexion with the climate, altitude, and soil. The plantations at Long Ashton are situated for the most part in the valley; throughout the village, and the district generally, numbers of old orchards are to be found, also large numbers of isolated trees of apple, pear and plum. The area is well wooded, elms predominating.

During the winter of 1920, as stated elsewhere (2), several old cider apple trees in land adjoining the plantations were cut down, and examination of the bark of these trees revealed the presence of large numbers of hibernating weevils.

There is little doubt that with the shelter afforded by old trees in close proximity to plantations, infestation is likely to occur year after year. As well as considering the plantations, therefore, it is necessary to take these old trees into account when arranging the annual programme of spraying operations.

Since the weevil is most active in bright sunshine the amount of sunshine in any particular district will influence, to some extent, the severity of attack.

With regard to distribution *Anthonomus pomorum* is recorded as generally distributed over the British Isles, but according to Collinge (3) it occurs in reduced numbers in Scotland. In Europe it is reported from France, Germany, Holland, Austria, Russia, Norway, Sweden, Denmark, Spain, Italy, Switzerland and Trans-Caucasia. It has also been recorded in the United States.

The weevil has been known as a pest for a considerable period, Knight(4) recording it over a century ago as a serious pest.

4. LIFE HISTORY AS OBSERVED AT LONG ASHTON.

In the spring when the buds are in the "delayed dormant" stage the weevil leaves its winter quarters and, crawling up the trees, proceeds to feed by piercing the opening buds and sucking the juices. This feeding

[1] Recorded in *Journal of Pomology* (Vol. III. Nov. 1922, p. 54).

goes on principally in bright sunshine, more weevils being found feeding at mid-day than at any other time.

After feeding for a few hours the insects retire to some shelter such as bark crevices and branch crotches and remain quiescent for a time. In this manner alternate feeding and resting go on for a period of about fourteen to eighteen days during which time copulation takes place. The only references to this spring feeding that have been found are one in *Pflanzenkrankheiten* by Sorauer, Vol. III, p. 535, and another in the *Zeitschrift für Pflanzenkrankheiten*, where a paper by Schulz(5) is abstracted and the following stated, "Ende März bezw. fliegen die Käfer in die Kronen von Apfelbäumen, müssen hier aber noch etwa 14 Tage den Inhalt von Knospen ausfressen, bevor die geschlechtsreif werden." By the end of this period the apple trees are in the "cluster-bud" stage and

Fig. 1. 1st instar larva of *Anthonomus pomorum*. Approx. × 50.

oviposition takes place, the eggs being laid inside the flower buds. After eight to thirteen days the larva hatches and proceeds to feed on the stamens and styles of the unopened flower bud. The infested bud does not open in the normal way but the petals remain closed and dome-shaped; they turn brown and dry, thus forming shelter and protection to the delicate larva (Fig. 1). These infested blossoms are known as "capped blossoms" and possess a very characteristic appearance; they begin to be noticed by the end of April. The larva feeds for about three weeks and when fully fed, usually by the end of the first fortnight in May, pupates. The pupal stage lasts for five to ten days according to the weather, at the end of which period the pupal coat is shed and the imago appears. The perfect insect remains inside the "capped blossom" for a day or two while its exo-skeleton is hardening up, and then cuts its way out. An exit hole is seen at *A* (Fig. C, Pl. XIX). Until the end of June or beginning of

July these young adults feed on the underside of the leaves of the apple and plum, the damage they do greatly resembling that done by one species of Tortrix moth[1]. Fig. D, Pl. XX, shows specimens of leaves upon which the weevils have fed.

Some authors maintain that the young adults hibernate without having fed. This is erroneous as shown above and, in addition, by an experiment carried out at Long Ashton in 1921, when about eighty capped blossoms were collected and the weevils on emerging were kept without food. These all died within about a fortnight after emergence.

By June 16th the young adults commenced seeking their winter quarters, a spell of cold weather about June 27th expediting matters.

The life cycle of *Anthonomus pomorum* may be summarised as follows:

Year	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
1920	—	—	—	—	—	—	—	—	AW	AW	AW	AW
1921	AW	AW	AA	AA, E, L	L, P, AA	AA	AW	AW	—	—	—	—

AW = Adult in winter quarters. AA = Adult active. E = Egg. L = Larva. P = Pupa.

A. The overwintering adult.

From September onwards through the winter the weevils were taken in a variety of winter quarters. In Worcestershire they were found under rough bark of plum trees, in curled up leaves both on the tree and on the ground, and in the surface soil; the most fruitful results, however, as shown by Lees (1), were yielded by the examination of the old grease bands. Under these the weevils were found in considerable numbers, for the most part, from 3 to 15 in close proximity to each other.

The grease bands were on both plum and apple trees and afforded practically the only available shelter, for the apples, especially, were very smooth barked; the plums, however, had split and creviced bark which would account for the weevils seeking them. Some few were found in the grease, having been caught while seeking winter quarters.

Later, in Gloucestershire, numbers were found under the bark of dessert pears and in the bark crevices of the younger orchard trees. In mid-winter specimens were found under the bark of isolated crab apple trees quite a mile distant from the nearest orchard; odd specimens were taken on elms and oaks near orchards.

At Long Ashton the principal winter quarters were rough bark of old cider trees, curled leaves, and under paper of grease bands. Subsequently numbers were found in the bark of the large branches from the fork

[1] This particular species fastens a leaf down with silken strands on to an apple and feeds, under this cover, on the under surface of the leaf.

upwards. The noticeable feature in reference to overwintering on the trees was that the weevils selected a position where there was pressure exerted on them from above; in no instance were they taken under the dry loose bark, their favourite places being not too dry and not too moist but always possessing this characteristic of pressure from above.

Certain other of the *Rhyncophora* were frequently associated with *Anthonomus* in its winter quarters. These included:

Anthonomus ulmi, De G.
Apion sp.
Cionus scrophulariae, L.
Cossonus ferrugineus, Clairv.
Hypera nigrirostris.
Mecinus pyraster, Herbst.
Orchestes sp.
Rhyncites coeruleus, De G.
Sitones lineatus, L.

(i) *Description of the Adult.*

The description given by Fowler(6) allows the weevil to be readily recognised. He describes it as follows:

"Pitchy black or fuscous black, clothed with ashy pubescence; head thickly pubescent, with an impression on forehead; rostrum long and rather slender, slightly curved, antennae long and slender, reddish fuscous at apex; thorax pitchy, sometimes rugo-piceous at sides, not much longer than broad, closely punctured, with rather scanty coarse white pubescence; scutellum thickly pubescent; elytra pitchy, sometimes pitchy ferruginous, with alternate denuded and white fasciae, the one behind middle, composed of whitish pubescence, being oblique and much the most conspicuous, punctured striae distinct and rather strong, interstices very closely punctured; legs more or less pitchy, but variable in colour, femora always more or less dusky, anterior pair with a very strong tooth, intermediate and posterior pairs with much smaller teeth. L. 3–4 mm."

(ii) *Sexual Differentiation in Anthonomus pomorum.*

The most reliable sex differentiating characters as given by Fowler are as follows:

"The males may be distinguished from the females by having the rostrum proportionately a little shorter, and, as a rule, duller, and the last ventral segment is sometimes somewhat variable in the sexes."

Owing to the great range of variation no dependence can be put on the proportionate size of male and female for many very small specimens, on dissection, proved to be females and numerous large ones males. The best distinguishing feature is the rostrum. At first it is by no means easy to decide from the rostrum the sex of a given specimen, but when the character is once definitely fixed and decided upon it becomes, with practice, quite an easy matter. The rostrum of the female is slenderly

spatulate, is darker than that of the male and has a more polished appearance.

The two specimens figured, Fig. E, Pl. XX, show the following differences in measurement of length of rostrum:

Rostrum 1·25 mm.	Total length 5·5 mm.
,, 0·75 mm.	,, ,, 4·5 mm.

Mouth parts. These are typical of the *Rhyncophora* and call for no special discussion, the accompanying figure (Fig. 2) being self-explanatory.

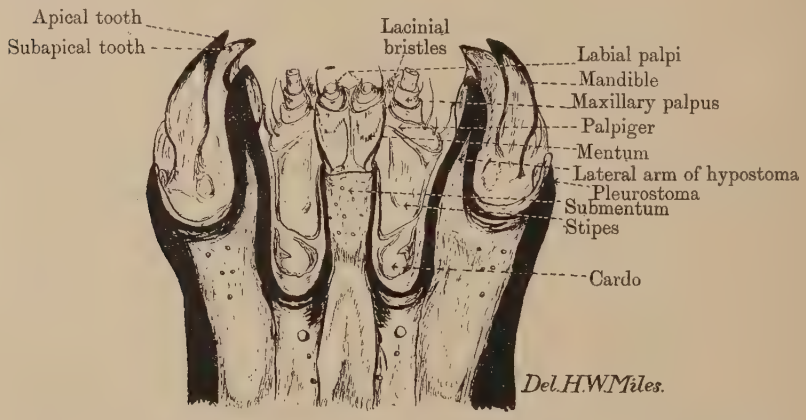

Fig. 2. Ventral aspect of mouth parts of *Anthonomus pomorum*. Highly magnified.

(iii) *Internal structure of Anthonomus pomorum*, Linn.

The Fat-body. (*Corpus adiposum.*) In *Anthonomus* this consists of a mass of tissue which almost completely envelopes the alimentary system. There is a ground tissue which contains large fat cells, and interlacing throughout the mass are fine tracheal fibrils. The colour varies from pale yellow, in the newly emerged adult, to a very rich yellow in the fully fed, and overwintering adult. The function of the fat-body is, apparently, fat storage. Obscure physiological processes are said to take place in the fat-body, some authorities connecting it with respiration, others with nutrition, while Marchal (7) associates it with the accumulation of urates.

The Alimentary System. The three primary regions are well differentiated; these are stomodaeum, consisting of mouth, pharynx, oesophagus and proventriculus; mesenteron, consisting of the ventriculus with the caecal glands; and proctodaeum consisting of the intestines with Malpighian tubes, colon, rectum and anus. These regions and their divisions

can all be readily located in the accompanying figure (Fig. 3). The proventriculus is an organ possessing a highly developed chitinous structure which adapts it for masticating purposes during that period when the insect feeds on leaf tissue. It consists of eight double rows of chitinous plates clothed with innumerable denticular ridges and associated with a system of circular muscles (Fig. 4). Packard (8) referring to the function of the proventriculus states, "It has been compared with the gizzard of

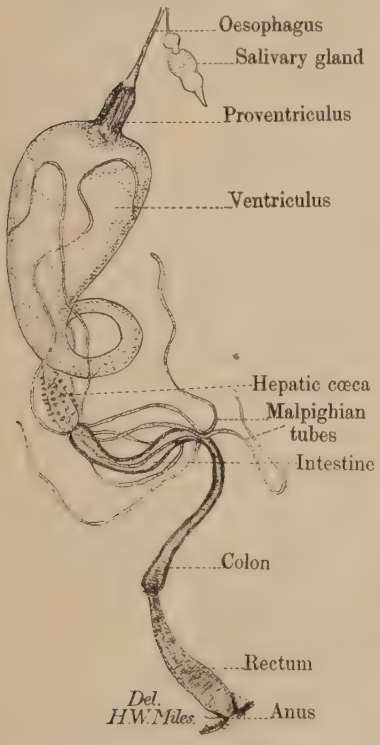

Fig. 3. Alimentary canal, *Anthonomus pomorum*. × 47½.

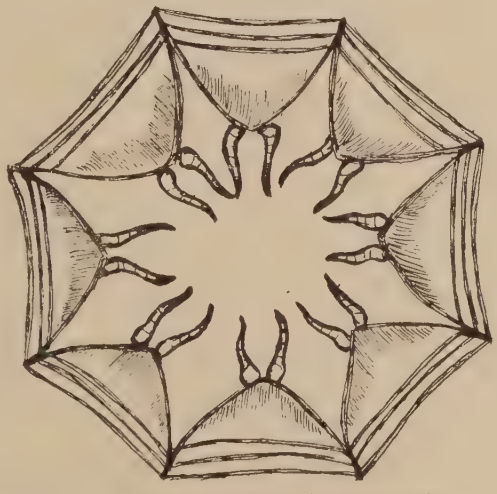

Fig. 4. Transverse section of Proventriculus, *Anthonomus pomorum*. Approx. × 400.

birds and is usually called by the German authors the chewing or masticating stomach (Kaumagen)." The posterior portion of the proventriculus projects for some little distance into the ventriculus. The mid-gut is divided into two distinct regions; the anterior is the ventriculus, a large receptacle for the macerated food which is lined with high columnar epithelial cells, and posteriorly is a constricted portion studded with caecal glands. These are short glandular bodies with large cells.

They are often very difficult to locate owing to their being intimately associated with parts of the fat-body and liable to come away with it on dissection.

At the junction of the mesenteron and the proctodaeum the Malpighian tubes enter; they are long, and much twisted, some running forwards and ramifying amongst the fat-body and around the ventriculus, and others running backwards and seeming to be connected by strands to the point where the colon runs into the rectum. The intestine is short and has slight circular and longitudinal muscles; the colon is glandular or absorbent, and the rectum is characterised by systems of well-developed circular and longitudinal muscles.

(iv) *The Reproductive Organs of Anthonomus pomorum.*

Examination of young adults soon after emergence shows the reproductive organs in an immature state, the testes in the male and the egg tubes in the female being quite undeveloped.

By the time the weevils seek their winter quarters the testes have developed and attained their normal size, but the ovaries are still undeveloped. The first noticeable change is apparent by early March, when the ovarian tubes show signs of development and by the time oviposition commences they are mature, as figured (Fig. 6).

The male reproductive organs, as figured (Fig. 5), consist of testes, *vasa deferentia*, *vesiculae seminalis*, common duct, or unpaired *vas deferens*, and penis with internal sac.

The testes are large yellowish bodies each divided into two main lobes, which in turn are again sub-divided. Each testis is covered by a thin membrane which carries the tracheal fibrils and connects the mass with the strands of the fat-body. From the testes the paired *vasa deferentia* lead into the common duct to the penis.

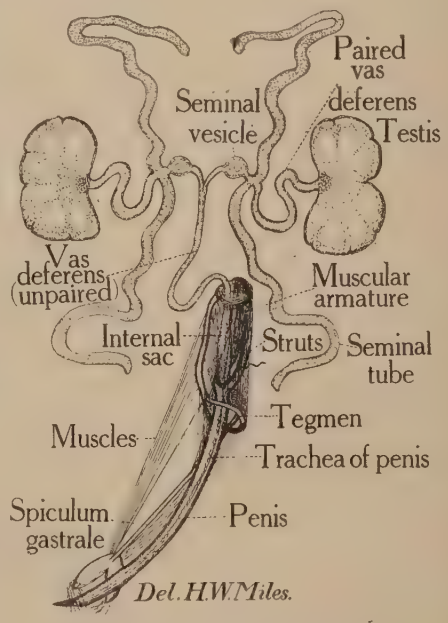

Fig. 5. Reproductive organs of male, *Anthonomus pomorum*. ×112.

Opening into the *vasa deferentia* are two seminal tubes and located immediately below their point of entry are the seminal vesicles. The unpaired *vas deferens* is a long coiled tube which leads, posteriorly, into the internal sac. This latter is located within the chitinous and muscular armature of the penis which consists of a sheath prolonged

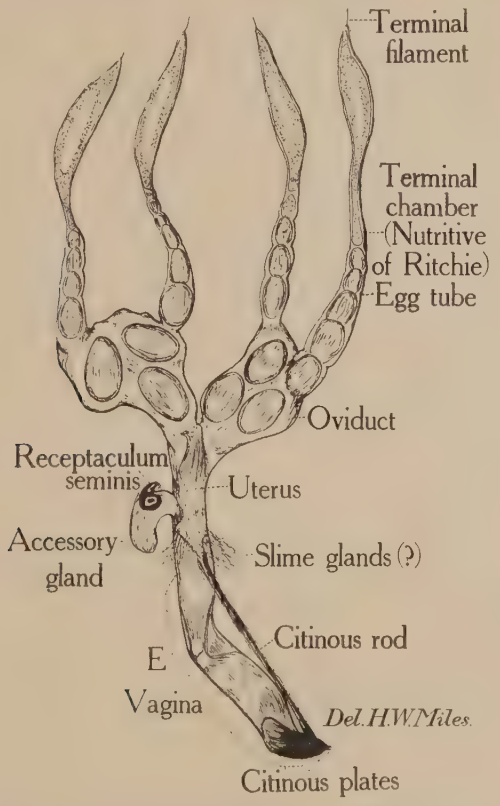

Fig. 6. Reproductive organs of mature female of *Anthonomus pomorum*. × 120.

anteriorly into two struts, a tegmen or ring with a tegminal strut, and a *spiculum gastrale*.

The female reproductive organs consist of two ovaries, one on each side of the body. Each ovary is composed of two ovarian tubes opening into a common oviduct, which leads into the uterus.

At the anterior end of each egg tube a terminal filament is located; this serves to suspend or connect the ovaries with the strands of the fat-body.

The apex of each ovarian tube consists of a nutritive chamber containing cells which furnish the eggs with nutritive material. In Fig. 6 the nutritive chamber is seen to become narrower towards the point where the eggs are being formed. In the oviducts the eggs are seen to be practically fully formed. The oviducts, at their posterior extremities, lead into the common chamber, the uterus, and its lower portion, the vagina. The *bursa copulatrix* runs into the uterus; it is connected with the *receptaculum seminis* and the accessory gland. Just below the junction of the *bursa copulatrix* with the uterus, a number of fine tubules enter the uterus; their function is unknown, but it is suggested that they may be analogous to the slime glands of many *Scolytidae*. At the posterior extremity of the vagina are two heart-shaped chitinous plates produced posteriorly into small rounded papillae. A chitinous rod runs from the median region of the uterus to the posterior extremity of the vagina. The function of the chitinous plates is suggested to be the guidance of the ovum at oviposition. At this latter operation the whole of the portion marked *E* (Fig. 6) is everted.

B. Feeding Habits and Oviposition.

In order to form some idea as to the feeding habits of *Anthonomus* on emergence from winter quarters specimens were taken into the laboratory and placed on twigs of apple which had been forced into growth in the greenhouse, the buds just showing green, a stage known to the grower as the "delayed dormant," or "bud-green." The weevils put on these twigs immediately crawled upwards to a terminal bud (Fig. B, Pl. XIX) where, tearing off and rejecting the surface tissue they forced the rostrum deep into the bud and, resting practically motionless, sucked the juices. From the punctures thus made drops of sap exuded. Later the expanded leaves showed evidence of the weevil having fed, in the form of circular holes. Where these marks occur in plantations they indicate the presence of the weevil at the bud-green stage. After feeding, the weevils went into hiding in cotton-wool and in crevices between the branches. In the same way weevils were found to feed on pear buds.

It was impossible to obtain in the plantations shoots of apple in the cluster-bud stage so early, but the foregoing data were sufficient to indicate that the weevils leave their winter quarters, feed for a shorter or longer period and return to shelter.

In 1921 the first appearance of weevils outside in the plantation was on March 24th when, about mid-day and in bright sunshine, numbers

were found crawling actively about the apple trees, piercing the opening buds in the process of feeding. Many were seen in copula. Feeding and resting took place until about April 6th, when ovipositing commenced, that is, about a fortnight after the first feeding and mating. The act of oviposition, as far as could be observed both in the plantations and in the laboratory under the binocular microscope, was as follows: First the pubescent outer tissue was torn off in small fragments and rejected. This was followed by a semi-rotatory and tugging movement with the feet firmly planted on the bud. The rostrum was then thrust downwards and the head rotated from side to side. The insect remained quiescent for a period varying up to 15 minutes, during which time, as revealed by subsequent examination, a cavity had been eaten in the anther lobe immediately below. The rostrum was then withdrawn and the female, reversing her position, after careful orientation, extended her ovipositor and thrust it down into the prepared aperture. In this position the female would remain for a short period, rarely longer than one minute, after which she withdrew the ovipositor and crawled either to the next bud where a similar procedure was adopted, or down into the cluster of bud-stalks to rest in a crotch. Theobald (9), referring to the act of oviposition, states that after the egg has been laid the female "turns round and pushes it far into the cavity with her snout....The hole is afterwards closed up...with saliva." In all the cases observed at Long Ashton the female was not observed to turn round and push the egg farther down into the cavity after having oviposited, and dissection of the buds selected for oviposition showed that the eggs were actually fixed into the tissue of the anther lobes in the cavities which had been eaten out for their reception (Fig. 7). As regards the closing of the puncture, though Curtis (10) also refers to such an action on the part of the female, in no instance was it observed by the writer

Fig. 7. Egg of *Anthonomus pomorum*, *in situ* in anther lobes of apple. × 60.

in 1921 or in 1922, but it was noticed that a sappy exudate from the pierced tissue readily filled the puncture and congealed, quite sealing up the entrance. The whole act of oviposition occupies from eighteen to twenty-five minutes[1] and in no instance was it observed to occupy three-quarters of an hour as stated by Curtis and Theobald.

While ovipositing was in progress many weevils were to be found on the trees resting in small crotches near blossom buds which showed signs, in the form of discoloured punctures, of their having fed. Some of these weevils, males and apparently unfertilised females, were collected and kept under observation in the laboratory, one specimen being observed to feed in a single puncture for upwards of two hours.

Feeding goes on for two or three weeks after the first sign of ovipositing and, towards the end of the period, the adults may be seen clinging lethargically to the buds or in small crotches on the trees; many of these were spent or unfertilised females.

One important point noticed was, that during the first bud-feeding stage and the early stages of oviposition the weevils would fold their limbs and drop from the buds at the least touch or jar, but about halfway through the ovipositing period no amount of jarring would induce them to fall.

C. The Egg Stage.

The eggs are broadly ovoid in shape, are densely white in colour and measure on an average ·7 mm. × ·5 mm. Where ovipositing took place in 1921 on April 5th the larvae hatched on April 18th, thus giving a period of 13 days for the duration of the egg stage. In 1922, however, eggs laid on April 28th hatched on May 6th, giving only eight days for the egg stage.

D. The Larval Stage.

On hatching, the larva is a soft white legless grub with the head comparatively large and black. The body is wrinkled into numerous folds and sparsely covered with hairs or bristles (Fig. 1). The spiracles are very minute and inconspicuous. No eye spots are present. The antennae are minute and rudimentary. Examination of numerous infested apple blossoms showed that the larvae commence feeding on the anther lobes; from these their depredations extend to the filaments and styles. Usually only one larva occurs in each capped blossom, but in 1921 in several instances two were found. The petals are gnawed partly through

[1] In many cases, under especially favourable conditions, it only occupied twelve minutes.

at their base, with the result that though they are attached to the receptacle they do not develop further but remain as a dome-shaped cap which turns brown and wrinkled as time goes on. With several moults, the larva continues feeding for fifteen to seventeen days, at the end of which period there remains nothing of the stamens or styles above the receptacle, the upper surface of which also frequently shows signs of the larva having fed. The full-fed larva presents the appearance shown in Fig. 8. It consists of a slightly retractile head, three thoracic segments and nine distinct body segments and bears numerous bristles. In the

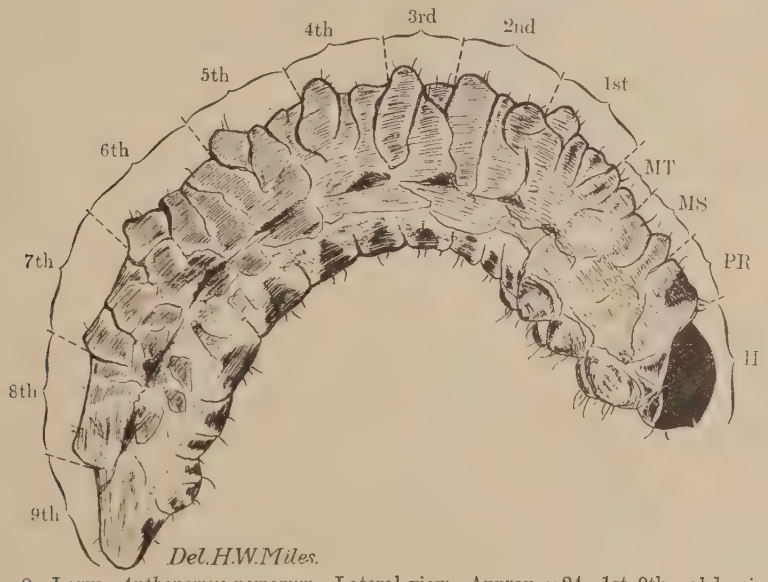

Fig. 8. Larva, *Anthonomus pomorum*. Lateral view. Approx. × 24. 1st–9th = abdominal segments. H = head, PR = pro-thorax, MS = meso-, MT = meta-thorax.

head can be discerned the labrum and clypeus on the sides of which are the mandibles. These are strongly chitinous and toothed, and below them lie the tri-segmented maxillary palpi. Posteriorly to the clypeus the epistomal and frontal areas can be seen. The frons is a triangular plate bearing several frontal bristles and behind it, occupying the greater portion of the dorsal area of the head, and bounded anteriorly by the frontal sutures, is the epicranium. This is divided into two portions by the epicranial suture, and bears several bristles (Fig. 9).

The thorax, consisting of pro-, meso-, and meta-thoracic segments, is readily located. The pro-thorax bears, dorsally, two darker, apparently

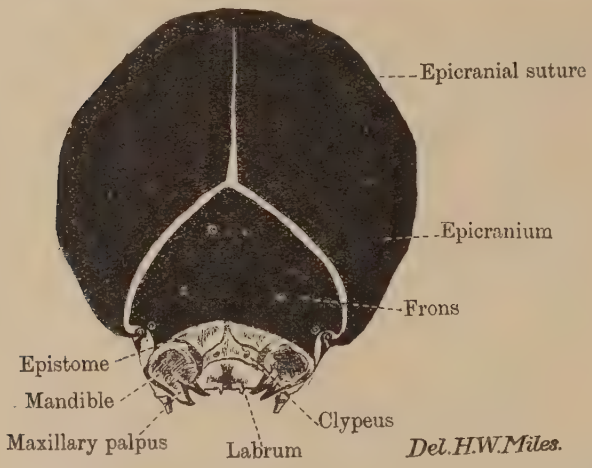

Fig 9. Head of larva, *Anthonomus pomorum*. Front view, highly magnified.

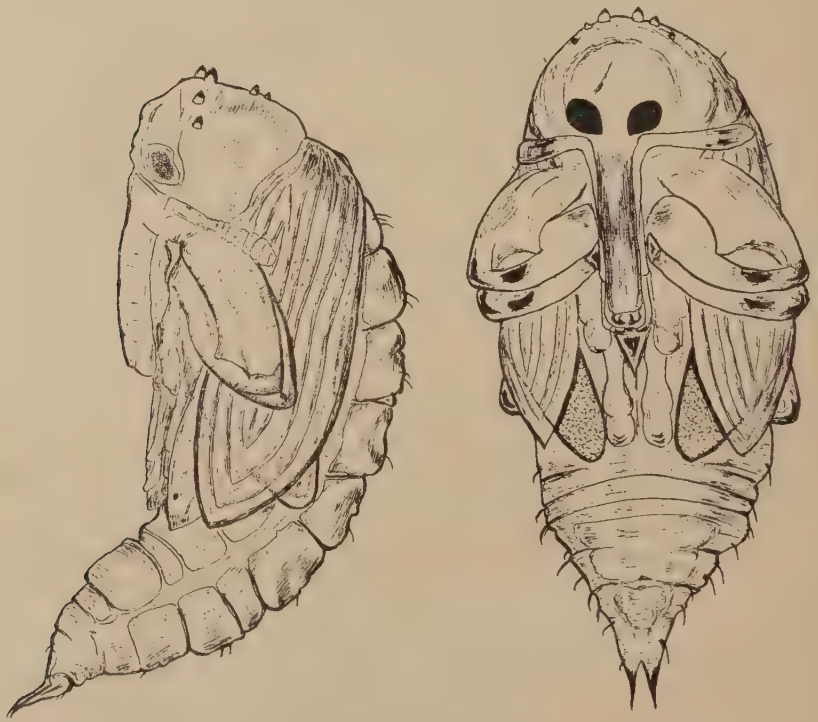

Fig. 10. Lateral view, Pupa *Anthonomus pomorum*. ×30.
Fig. 11. Pupa *Anthonomus pomorum* darkening up prior to emergence. Ventral view ×30.

chitinous areas. The other thoracic segments are divided into prescutal and scutellar lobes. The abdominal tergites show division into prescutal, scutal and scutellar lobes, of which the last named are very prominent. The divisions between the lobes are deeply cleft, which characteristic, in the sternites and pleurites, renders difficult the location of the spiracles. There are nine spiracles on each side, the first being located in the pro-thoracic segment and the remainder in the first to the eighth abdominal segments.

The Change from Larva to Pupa.

The fully-fed larva, which measures about 6 mm. in length, is superficially dirty white or slightly creamy in colour. Through the outer skin the brownish food contents are visible, with the result that the larva may present a very mottled appearance. At the cessation of feeding, processes go on internally whereby digestion is finished, and the indigestible residue defaecated. The larva which is now a clean light yellow colour, remains quiescent and, after about two days, pupation takes place and the larval skin is shed.

E. The Pupal Stage.

The general characters of the pupa are shown in Figs. 10 and 11. The pupa is soft, fleshy, and clothed laterally and dorsally with numerous stout bristles. The legs, elytra, wings, antennae and rostrum are folded in characteristic fashion, and the whole is swathed in a thin transparent pupal coat. The pro-thorax bears numerous sharp conical tubercles; it is suggested that these may be used to break the pupal coat at emergence. The ninth abdominal segment bears two stout caudal spines which are used to effect any movement. The length of the pupal stage is from five to eight days, or even longer.

Development and Emergence.

The first sign of development in the pupa is the darkening of the eyes which occurs, faintly, after about two days. By the third day the eyes are distinctly noticeable, the mandibles are brownish, the rostrum denser and the membranous wings are smoky. Immediately before emergence of the weevil the eyes, rostrum, and antennae are dark and the dorsal area of the pro-thorax and the margins of the meso- and meta-thoracic tergites are also denser. The tarsal claws are next to darken, followed by the articulations of the limbs, and the tip of the abdomen; still later the femora darken and the femoral teeth become very distinct.

At emergence the pupal coat splits along the line of pro-thoracic tubercles and forward down the rostrum. The clubs of the antennae are forced apart, the forelegs are slightly extended, and periodic abdominal movements may be seen. These rotary abdominal movements result in the pupal coat being forced backwards. The clubs of the antennae and the tip of the rostrum remain attached to the pupal coat for some short time; finally, however, they are freed and only the posterior tarsi retain the coat until the caudal portion comes away. The whole process takes about three-quarters of an hour. After emergence the legs are stretched and moved, the membranous wings extend to their full length and are then folded, the elytra falling into place above them. The abdomen shortens until the pygidium is drawn beneath the elytra. The chitinous structures harden up rapidly and the elytra assume their dark colour above and below the "V" mark.

F. The Young Adult.

On emergence from the capped blossoms the young adults of *Anthonomus* are very active and will fly slightly more readily than do the overwintered adults. They feed on the petals of opened blossom and on the under surfaces of the leaves. In the latter case the upper epidermis is left intact. In captivity both apple and plum foliage were used as food.

Dissection of a male specimen three days after emergence showed the ventriculus full of macerated food and all the intestinal portion much distended. The testes were very small and undeveloped. The chitinous exo-skeleton and the elytra were soft and fragile as distinct from the tough coats of the overwintered adult. During the period at the end of May and early June various substances were used in an endeavour to find out if the young adults could be attracted for control purposes, but no success was obtained. As in the spring feeding of the overwintered adult so in the summer feeding of the newly emerged adult, it was noticeable that alternate periods of feeding and resting occurred, the weevils seeking any available cover after feeding, some re-entering the capped blossom, others crawling into crotches or bark crevices or into the herbage beneath the trees, where cover-cropping was followed.

The first emergences of new weevils in captivity were noticed on May 13th; by June 27th the fat-bodies were well developed and yellow, and the testes practically full size and yellow. On June 25th a specimen, taken from under the bark out of doors, was dissected; the stomach was empty, the fat-body was well developed but pale and the testes were practically of full size and yellowing.

From this evidence it can be concluded that the young adults, in an average season, will be fully fed by the end of June or beginning of July and from this period onwards will be seeking winter quarters. The earliest date of finding weevils in their winter quarters, under paper bands tied on the trunks of trees, was June 16th. In order to obtain some data as to the movements of the weevils towards winter quarters, three bands were placed on trees on June 10th. These were examined with the result that weevils were collected as follows:

June, 34; July, 25; August, 7; September, 12; October, 5.

This indicates that the majority of weevils seek winter quarters in June and July.

5. FACTORS LIMITING THE DISTRIBUTION AND EXTENT OF *ANTHONOMUS POMORUM*.

The chief factors limiting the distribution of *Anthonomus pomorum* are:

A. The distribution and extent of its host trees which are: (*a*) apple, (*b*) pear, (*c*) crab apple.

B. Climatic conditions.

C. Natural enemies: birds, insects and fungi.

A. DISTRIBUTION AND EXTENT OF HOST TREES.

In the west of England apples and pears are cultivated extensively in an area consisting of Somerset, Gloucester, Hereford, Worcester and parts of Monmouth, Dorset, Oxford and Wiltshire, and throughout this area the conditions are such as to favour the persistence of the weevil, viz. a mixed tenure composed of intermingling old cider orchards, and plantations of younger trees of choice varieties. The old cider orchards furnish ample winter quarters, and in spring numbers of overwintered adults spread to adjoining plantations, where they oviposit. Older orchards are therefore a source from which migration, to a greater or less extent, will constantly take place. Isolated trees are also infested, and during the late winter and early spring of 1920–21 hibernating adults and "capped" blossoms were found on crab apple trees, some of which were over a mile from the nearest orchard.

B. CLIMATIC CONDITIONS.

As regards the climatic factor, the main influence is felt in spring, during the latter part of March and on to the end of April. Bright sunshine and warm days make the weevil leave its winter quarters and seek

the buds for food. Following the first feeding period there is a lapse of from one to two weeks, pending the ripening of the ovaries for oviposition; and if, during this period, bright warm days continue, the development of the blossom-buds is very rapid and, by the time the female is ready to lay her eggs, a considerable number of buds may have passed the ideal stage, the cluster-bud stage, and others have just sufficiently developed as to open during the ten days or so of incubation, and the young larvae having no protection, perish, resulting in a minimum of loss through weevil attack.

On the other hand, when the period of blossom-bud development is protracted or checked by cold and dull spells of weather, the weevils have their period for ovipositing considerably lengthened, and so the loss due to capped blossom is considerably increased.

C. Natural Enemies.

(i) *Birds.*

Undoubtedly birds are of considerable importance in keeping in check the apple-blossom weevil. Though no special observations in this connexion were made at Long Ashton, from time to time several species have been reported as definitely destroying *Anthonomus* sp.; these are—woodpeckers, tits and sparrows. Theobald (9) states that woodpeckers are of use, while Collinge (11) records finding specimens of *Anthonomus pomorum* amongst the stomach contents of the blue-tit. With regard to sparrows, Maarschalk (12) gives an instance of their pecking open capped blossoms and destroying the larvae; this was in Holland, but so far no similar record has been made in England. Newstead (13) states, in reference to the marsh-tit, that it "is responsible for the capture of some specimens of the apple blossom weevil." In Gloucestershire it was particularly noticeable that, in the spring of 1921 when *Anthonomus pomorum* was egg-laying, the nut-hatch (*Sitta caesia*, Wolf.) was very active, and spent several weeks feeding in the orchards.

(ii) *Insects.*

Other natural enemies of *Anthonomus* occur among insects and include one species from the Thysanoptera, *Haplothrips tritici* (Kurd.), a predaceous thrips, which is recorded by Kurdjumov (14) as feeding, *inter alia*, on the larvae. A number of species of the order Hymenoptera are parasitic on the larva and pupa of the weevil.

Imms(15) gives a summary of the species recorded from *Anthonomus pomorum*; this includes:

1. *Pimpla pomorum*, Ratz.
2. *Pimpla examinator*, Fab.
3. *Pimpla graminellae*, Grav.
4. *Pimpla sagax*, Stg.
5. *Apanteles lacteus*, Nees.
6. *Apanteles impurus*, Nees.
7. *Campolex latus*, Ratz.
8. *Meteorus ictericus*, Nees.
9. *Habrocytus fasciatus*, Thoms.

Only one of these species was observed at Long Ashton, viz. *Pimpla pomorum*, Ratz., specimens of which were submitted to Dr Imms who forwarded them for identification to Mr Claude Morley.

The larvae of this species are ecto-parasitic in habit, feeding on the larva and pupa of the host. In his summary of "Observations on *Pimpla pomorum*," Imms states, "The larva of this Ichneumon is composed of a definite head and thirteen trunk segments; no caudal process or anal vesicle is present. Ten pairs of spiracles are evident, but the pair situated on the second segment is minute and vestigial. Pupation takes place within a slight silken cocoon spun within the cavity of the unopened apple buds." The adults emerge about twenty-three days after the cocoons are spun. Where the *Anthonomus* larva is attacked, the parasite is usually located, as shown by Imms (*op. cit.*), on its dorsal side, but in the case of the pupa the anal extremity is selected. Half-devoured pupae of *Anthonomus* are very common in the capped blossom, and Imms, referring to these, states that, since the remaining portion shows no signs of discoloration or putrefaction, "it appears not unlikely that the parasitic larva may inject some kind of secretion into the tissues of its host, serving as an antiseptic arresting decomposition."

With regard to the efficiency as a parasite, of this species, at Long Ashton the parasitism worked out at about 5 per cent., twelve specimens of *Pimpla* being reared from 238 capped blossoms.

Imms gives an instance of 27·4 per cent. parasitism in buds examined in 1916 and quotes Decaux as rearing and liberating about 250,000 parasites from the infested buds collected from 800 apple trees.

The specimens reared at Long Ashton included both males and females. The female has been known since 1848 when Ratzeberg described it, but few records of the male exist and of these the first English specimens were recorded from Cambridgeshire in 1916 by Imms for whom a full description of the sex was drawn up by Morley.

The occurrence of males in widely separated areas like Cambridgeshire and Somerset suggests a probable fairly wide distribution of this sex.

In January, 1921, an endo-parasitic larva was taken from a specimen of *Anthonomus pomorum* sent in from Worcestershire. This was submitted for examination to Dr Imms who reported on it as follows:

"Your larva is most certainly that of an Ichneumon in the first instar —the caudal prolongation is a common feature among endo-parasitic *Ichneumonidae*."

This parasite was located in a male weevil between the testes and high up towards the dorsal region of the abdomen; the testes were very pale in colour and the fat-body was tinged with brown as distinct from its fresh yellow colour in healthy specimens.

No adults of internal parasites were reared from overwintered weevils.

(iii) *Fungi.*

In the spring of 1921 examination of some old orchard trees showed numbers of *Anthonomus* dead and heavily infected with a fungus which was apparently a species of *Isaria*. The mortality was about 4 per cent. No other fungal parasites have been observed.

6. SUMMARY.

1. The life history of the Apple-Blossom Weevil, *Anthonomus pomorum*, a serious pest in apple-growing districts, has been worked out and is herein described.

2. The adult, of which a full description, including that of the alimentary and genital systems, is given, spends the winter hibernating in or near apple orchards.

3. In spring the adults leave their winter quarters and, prior to mating, feed by piercing the fruit buds and sucking the juices.

4. Egg laying is performed when the development of the buds is in the stage known as "Cluster-bud." The egg is thrust into the centre of the young flower by the ovipositor of the female.

5. The stages, oval, larval, and pupal are described followed by a discussion of the habits of the young adult which is shown to feed on the under surfaces of the leaves.

6. Natural enemies, including birds, insects and fungi, are shown to play an important part in limiting the extent and distribution of the weevil.

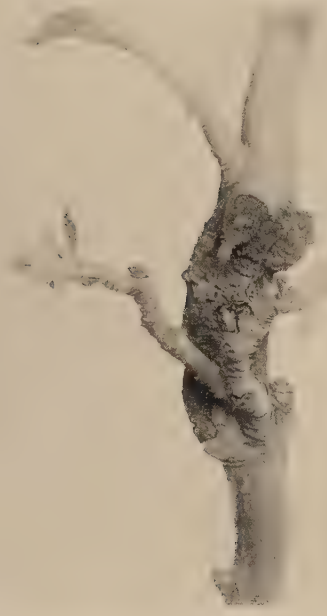

Fig. A

Fig. B.

Fig. C.

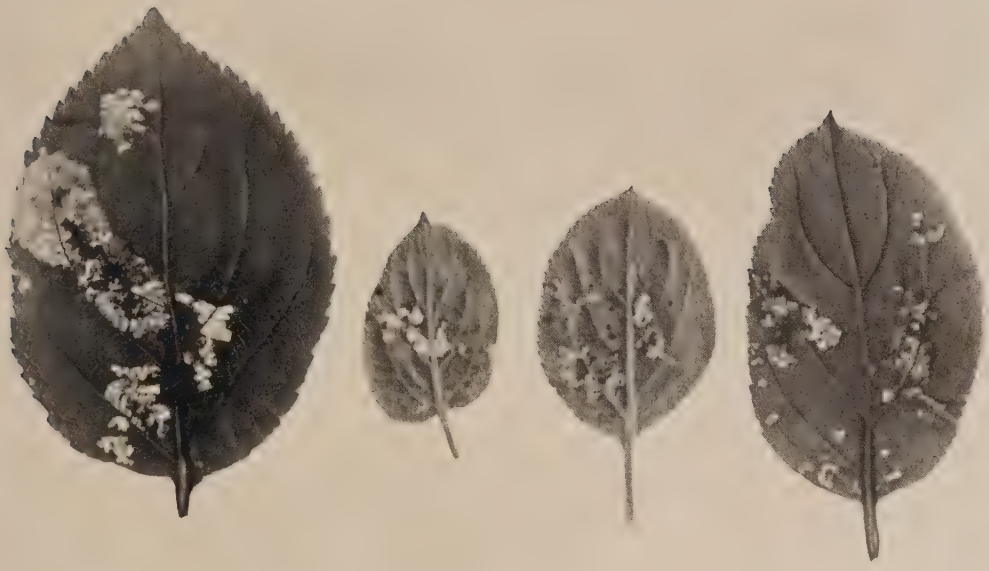

Fig. D.

Fig. E.

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EXPLANATION OF PLATES XIX AND XX.

Fig. A. A type of canker spot often selected by *Anthonomus* as winter quarters. ($\times \frac{1}{2}$.)

Fig. B. *Anthonomi* feeding on apple buds in spring. The buds are in the "delayed dormant" stage. Note weevils resting in crotches. (Nat. size.)

Fig. C. "Capped" blossom. A = exit hole made by young adults on emergence. (Nat. size.)

Fig. D. Leaves of apple damaged by the feeding of young adults of *Anthonomus pomorum*, L. (Nat. size.)

Fig. E. Adults of *Anthonomus pomorum*, showing difference in size and in length of rostrum. ($\times 3{\cdot}8$.)

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A STUDY OF A CAUSE OF "BLOWING" IN TINS OF SWEETENED CONDENSED MILK

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The work described below had its origin in the fact that a tin of sweetened condensed milk was found to be so badly "blown" that it burst on handling. In order to seek the cause of this defect, portions of the contents of the tin were inoculated into liquid media of various kinds and incubated. Gas production occurred in beer-wort and in sterilised milk to which cane sugar had been added. Microscopical examination demonstrated the presence of numerous yeast cells in those tubes which had shown fermentation. Plates were then made on beer-wort agar and yeasts of two types were found. The one gave heavy white colonies consisting of round cells (described as "*A*" in the text); the other type of colony grew more slowly, was translucent, and consisted of oval cells (described as "*B*" in the text). Both these yeasts were capable of producing gas in sterilised milk containing a high percentage of cane sugar. These facts having been ascertained, a more detailed study of each type was carried out with cultures obtained from single cells. Hansen's(3) method, using a Böttcher chamber, was adopted for the isolation of *A*, and Barber's(1) method for *B*.

Morphology and Cultural Reactions of Yeast *A*.

This type consisted of round cells of diameter 5–6 μ. In old cultures a number of larger cells were found, some of which attained a diameter of 8·5 μ. Spores have not been obtained either on thin layers of gelatine or on gypsum blocks.

A streak culture of this yeast on beer-wort agar gave a heavy white growth which tended to become brownish and "granular" in old cultures. In beer-wort agar stab cultures there was growth throughout the stab and good growth at the surface. In beer-wort vigorous fermentation was set up and frequently a ring of scum was formed around the sides of the tube.

This yeast readily fermented Saccharose, Dextrose and Laevulose, but neither Lactose nor Maltose. There was a doubtful fermentation of Galactose. No fermentation was set up in milk, but when cane sugar was added, even in a concentration of 70 per cent., gas production and coagulation of the milk occurred.

In order to test whether this yeast was able to cause "blowing," growth from cultures on beer-wort agar was introduced into tins of sweetened condensed milk, which were then securely re-sealed and placed in an incubator at 22° C. At the end of ten to seventeen days the tins were slightly "blown," and one began to leak in five weeks. A similar tin which had been inoculated with a "gassy" culture in milk + 60 per cent. cane sugar showed only very slight "blowing" at the end of five weeks. Control tins of condensed milk kept under exactly the same conditions remained normal throughout the whole of these periods. When one of the blown tins was opened after three months' storage at ordinary room temperature, the contents were found to be still fluid, showing no evidence of "lumps." A considerable amount of gas had been dissolved in the milk which became "frothy" when the pressure was released. There was a slightly alcoholic taste and smell.

Morphology and Cultural Reactions, etc. of Yeast *B*.

Young cultures of this type consisted of oval cells of length 3–4·7 μ and breadth of 1·5–2·7 μ. These sometimes attained a length of 5 μ and a breadth of 3 μ in older cultures. The cells reproduced themselves by rapid budding, and since the young cells tended to hang together, small colonies were frequently found in liquid cultures. Spores have not been demonstrated either on gelatine or gypsum blocks.

A streak culture on beer-wort agar showed a light, white growth which became brown in old cultures. In stab cultures there was growth throughout the stab, but practically none at the surface. In beer-wort a vigorous fermentation was set up, but no scum was formed.

Saccharose, Dextrose and Laevulose were rapidly and vigorously fermented, but not Maltose or Lactose. A slight fermentation was produced occasionally in Galactose. In milk there was no fermentation, and very scanty growth, but in milk containing cane sugar there was rapid fermentation accompanied by coagulation of the milk. High concentrations of sugar seemed to have no inhibitory effect, for the yeast was able to cause vigorous fermentation in milk + 70 per cent. cane sugar.

When, as in the case of *A*, this yeast was inoculated into tins of sweetened condensed milk, which were then placed in an incubator at 22° C., a rapid fermentation ensued. The tins inoculated from cultures of *B* on beer-wort agar were found to be "blown" in four days and one burst in seventeen days. A tin inoculated from a "gassy" culture in milk + 60 per cent. sugar was "blown" in seventeen days. One of the "blown" tins was opened after three months' storage at ordinary room temperature. There was a strong rush of gas on piercing the lid. The contents had a distinctly etherial odour. Coagulation of the milk had taken place, and for about half the depth of the tin the milk was almost solid. The "lumps" had a stinging and slightly acid taste. The changes produced in the contents of the tins were much greater than in the case of *A*.

A study of the results published by workers in other countries suggests that defects in sweetened condensed milk having an origin similar to that described above are of frequent and wide-spread occurrence. Pethybridge (5) has demonstrated that a round and an oval yeast were the cause of "blowing" in tins of Irish condensed milk. As far as the data given allow of comparison these two types are very similar to those described ("*A*" and "*B*") although the oval appears to be somewhat smaller. Hammer (2) has isolated from American sweetened condensed milk an oval yeast which was proved to be the cause of "blowing" in the tins, and which, in its cultural reactions closely resembled *B*. It was, however, considerably smaller, and the cells showed no tendency to hang together in groups. In this connection it may be mentioned that, whereas the measurements for *B* recorded in the text were made on unstained cells, stained preparations give figures which correspond with those of Hammer. Knudsen (4) has already made this observation. Further, this yeast, *B*, rarely forms colonies when grown in milk containing a high percentage of sugar, but appears mainly as isolated cells, or cells having a bud attached. Knudsen (4) has described two types of yeast; one of these was round, and was isolated from "blown" tins of both Danish and Dutch sweetened condensed milk; the other was oval and was obtained from "gassy" Dutch sweetened condensed milk. By the courtesy of Professor Orla-Jensen the author has had the opportunity of making comparative tests with these, in conjunction with Knudsen, at the Polytechnic College, Copenhagen. There seems to be no doubt that the two types are identical with those described in this paper.

Thermal Death Point.

A study of the thermal death point of these yeasts seemed to be of considerable practical importance. Experiments were carried out on cells from cultures on beer-wort agar which had been kept at 22° C. for two days.

The cells were washed off in sterile saline, and quantities of 10 c.c. of this emulsion heated in test tubes to various temperatures, by plunging the tubes into water which was kept at a temperature 2–3° C. above that required. After heating, the tubes were plunged into cold water. Other experiments were carried out in which the tubes were kept at given temperatures for ten minutes.

To determine the vitality of the cells, measured quantities of the heated emulsions were inoculated into various media, *e.g.* beer-wort agar plates, beer-wort agar slopes, beer-wort, and milk + 60 per cent. cane sugar. The number of colonies on the plates, and the presence or absence of growth in the various media were noted.

Both *A* and *B* were able to survive an exposure to 50° C. for ten minutes while *B* was unable to resist 55° C. for ten minutes. Occasional cells of *A* were found to resist this exposure, but no growth was demonstrated after heating at 55° C. for fifteen minutes, or at 60° C. for ten minutes.

The emulsions which had been momentarily heated to given temperatures showed somewhat erratic results. The majority of the cells of both *A* and *B* were killed by momentary exposure to a temperature of 70° C. Nevertheless it has been found that occasionally cells of *A* survived a temperature of 72° C. and cells of *B* even 76° C.

It was felt, however, that it was very difficult to adjust the temperatures with any great degree of accuracy when such rapid methods of heating were adopted. A second series of experiments was carried out in which the tubes were placed in cold water and the temperature of the whole water bath gradually raised. The final temperature could thus be accurately controlled.

Second Series of Experiments. In order that the heating might be carried out under conditions which might occur in the condensed milk industry the cells were heated in milk or in cane sugar syrup. The cultures used had been grown under varying conditions for varying periods of time. Seven experiments were carried out in this series.

In the first four experiments, cultures on beer-wort agar were employed. For Exps. 1 and 3, the culture had been incubated at 22° C.

for two days, for Exp. 2, the culture had been kept at 22° C. for nine weeks, for Exp. 4 the culture had been incubated at 22° C. for two days, and kept at room temperature for nineteen weeks. In each case the cells were washed off in sterile saline, and from these emulsions measured quantities were added to tubes containing 9 c.c. of sterile 50 per cent. syrup sugar, or of sterile milk, and heated to the temperatures shown in the table. 1 c.c. of syrup was then added to each tube of milk and 1 c.c. of milk to each tube of syrup, and the tubes incubated at 22° C. The presence or absence of gas was noted.

The rate of heating of the emulsions of the organisms was kept as nearly constant as possible throughout the series. Table I gives the actual figures.

Table I.

			Time taken to raise temperature of emulsion				
Expt.	Emulsion	Initial temp.	From init. temp. to 55° C.	55–65° C.	65–73° C.	73–85° C.	85–100° C.
1 and 2	Milk	14° C.	17 mins.	12 mins.		7 mins.	9 mins.
	Syrup	14° C.	17 ,,	14 ,,		8 ,,	18 ,,
3 and 4	Milk	12° C.	18 ,,	15 ,,		8 ,,	11 ,,
	Syrup	12° C.	18 ,,	14 ,,		8 ,,	10 ,,
5 and 6	Milk	22° C.	13 ,,	6 mins.	6 mins.	10 ,,	9 ,,
	Syrup	22° C.	12 ,,	6 ,,	5 ,,	6 ,,	9 ,,
7	Syrup	21·5° C.	14 ,,	8 ,,	7 ,,	9 ,,	7 ,,

During the process of heating, the temperatures of the syrup and the milk in the inoculated tubes were taken as being the same as that of control tubes containing thermometers. The tubes were cooled by immediate plunging into cold water.

Exps. 5, 6 and 7 were carried out in a slightly different manner, since it was considered advisable to study the growth of the heated cells in milk containing a high percentage of sugar. The tests on the vitality of the cells were, therefore, made in milk to which 60 per cent. cane sugar had been added.

In Exp. 5, a culture on beer-wort agar which had been incubated at 22° C. for four days, and kept at 37° C. for seven days, was used. Emulsions were made in sterile whole milk, and sterile 50 per cent. cane sugar syrup, and quantities of 3 c.c. heated to the temperatures shown in Table I. After heating and cooling, 1 c.c. from each sample was inoculated into milk + 60 per cent. sugar and incubated at 22° C.

For Exp. 6, a culture on nutrient agar, to which 60 per cent. cane sugar had been added, was incubated at 22° C. for four days and kept at

37° C. for seven days. An emulsion was made in 50 per cent. cane sugar syrup, and 5 c.c. quantities were heated in the usual manner. From these, inoculations were made into milk + 60 per cent. sugar, and the tubes incubated at 22° C.

Exp. 7 was devised to investigate whether these yeasts were likely to remain alive for a prolonged period, or to develop an increased resistance to heat, when stored on sugar. Cane sugar was placed in flasks and sterilised by autoclaving, so that it formed a very thick syrup containing numerous sugar crystals. As much as possible of the supernatant liquid was removed from a vigorous culture of each yeast in beer-wort, and the residues, consisting mainly of yeast cells, placed on this sterilised sugar, and kept at 30° C. for three weeks and then at room temperature for seven weeks. The cells were then washed off with a sterile sugar syrup and heated as in previous experiments. Inoculations were made into milk + 60 per cent. sugar and the tubes incubated at 22° C.

In every case except Exp. 5, where the fact that the emulsion had been made in milk rendered it impossible, the number of cells introduced into each tube was determined by the Haemocytometer method.

Results.

The results obtained are shown in Table II which demonstrates:

1. That under the conditions of these experiments *B* was killed at a temperature between 55° and 65° C., whilst *A* was able to resist a temperature of 73° C. and, on one occasion, even 85° C. Exps. 1, 2, 3, 5, each show that *A* had a greater heat resisting capacity than *B*.

2. In Exps. 1, 2, 3, 5, cells of *A* which were heated in milk resisted a higher temperature than those which were heated in syrup. In Exp. 4, no such difference was found; in fact, cells of *B* which had been heated in milk were killed at a temperature of 55° C., whilst those heated in syrup were still capable of growth. In this case, however, the culture used was known to be very feeble.

3. On the whole, the results indicate that *B* is less resistant to heat than *A*, and that its thermal death point is but slightly influenced by varying external conditions. On the other hand, there are indications that the thermal death point of *A* is influenced by the nature of the heating medium.

Table II.

Exp.	Culture	Emulsion	Number of heated cells introduced into each tube	Temperatures (° C.) to which emulsions were heated: Unheated	55	65	73	85	100
				A. (Round cells)					
1	Culture on B.W.A. 2 dys. at 22° C.	Milk	600,000	+	+	...	+	−	−
		Syrup	,,	+	+	...	−	−	−
2	Culture on B.W.A. 9 wks. at 22° C.	Milk	800,000	+	+	...	+	+	−
		Syrup	,,	+	+	...	−	−	−
3	Culture on B.W.A. 2 dys. at 22° C.	Milk	5,400,000	+	+	...	+	−	−
		Syrup	,,	+	+	...	−	−	−
4	Culture on B.W.A. 2 dys. at 22° C. 19 wks. at R.T.	Milk	12,500,000	+	+	...	−	−	−
		Syrup	,,	+	+	...	−	−	−
5	Culture on B.W.A. 4 dys. at 22° C. 7 dys. at 37° C.	Milk	?	+	+	+	+	−	−
		Syrup	2,970,000	+	+	−	−	−	−
6	Culture on N.A. + 60 % 4 dys. at 22° C. 7 dys. at 37° C.	Syrup	2,100,000	+	+	+	−	−	−
7	Cells on sugar 3 wks. at 30° C. 7 wks. at R.T.	Syrup	520,000	+	+	−	−	−	−
				B. (Oval cells)					
1	Culture on B.W.A. 2 dys. at 22° C.	Milk	1,760,000	+	+	...	−	−	−
		Syrup	,,	+	+	...	−	−	−
2	Culture on B.W.A. 9 wks. at 22° C.	Milk	2,200,000	−	−	...	−	−	−
		Syrup	,,	−	−	...	−	−	−
3	Culture on B.W.A. 2 dys at 22° C.	Milk	23,400,000	+	+	...	−	−	−
		Syrup	,,	+	+	...	−	−	−
4	Culture on B.W.A. 2 dys. at 22° C. 19 wks. at R.T.	Milk	26,600,000	+	−	...	−	−	−
		Syrup	,,	+	+	...	−	−	−
5	Culture on B.W.A. 4 dys. at 22° C. 7 dys. at 37° C.	Milk	?	+	+	−	−	−	−
		Syrup	2,100,000	+	+	−	−	−	−
6	Culture on N.A. + 60 % 4 dys. at 22° C. 7 dys. at 37° C.	Syrup	1,380,000	+	+	−	−	−	−
7	Cells on sugar 3 wks. at 30° C. 7 wks. at R.T.	Syrup	2,660,000	−	−	−	−	−	−

+ = Presence of gas. − = Absence of gas. B.W.A. = Beer-wort agar.
N.A. + 60 % = Nutrient agar + 60 % cane sugar. R.T. = Room temperature.

Summary.

1. Two types of yeasts, *A*, round cells, *B*, oval cells, were isolated from a "blown" tin of sweetened condensed milk.

2. Both types were able to reproduce similar "blowing" when inoculated into tins of sweetened condensed milk.

3. The oval type was considerably more rapid and vigorous in its action than the round, but was less resistant to heat and drying.

4. These yeasts are compared with those isolated by Pethybridge from Irish, by Hammer from American, and by Knudsen from Danish and Dutch, sweetened condensed milk.

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A CRITICAL ENQUIRY INTO THE ALLEGED FIXATION OF NITROGEN BY GREEN ALGAE[1]

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(With Plates XXI and XXII, and 3 Text-figures.)

I. INTRODUCTION.

Since the investigations of Lawes and Gilbert (14,15) and of Hellriegel and Wilfarth (10) on the higher plants and of Kossowitsch (12) on the algae, it has been generally believed that green plants are not able by themselves to assimilate atmospheric nitrogen. Claims have been made from time to time, however, that certain chlorophyll-bearing organisms (*e.g.* algae, mosses and even higher plants) are able to derive their nitrogen directly from the air.

As far as algae are concerned, the literature on the subject up to 1914 has been adequately summarised and discussed by Schramm (23); a detailed consideration of this earlier work is thus rendered unnecessary in the present paper, but references to the more important of these papers are included in the bibliography (3,7,8,9,13,16,19,22). The position up to and including Schramm's work may be briefly summarised as follows. The greater number of the investigations had been carried out on cultures of algae which were impure. In several of these investigations nitrogen-fixation had been established, but, in considering their bearing on the question of the ability of algae themselves to fix nitrogen, these results may be dismissed, since it is impossible to say whether the fixation was effected in part by the algae or wholly by the associated bacteria. This does not preclude the possibility that the algae are indirectly concerned in the process of nitrogen-fixation by bacteria; indeed, from the work of Kossowitsch (12) and others (1,2,5,20,21), it is extremely probable that algae and nitrogen-fixing bacteria work together in a definite association, the algae stimulating the activity of the bacteria and enabling them to fix greater quantities of

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nitrogen than they could do alone. Heinze (9) claimed that he had obtained evidence of nitrogen fixation in a culture of *Nostoc* contaminated with a species of *Streptothrix* which alone had given no evidence of such fixation; he therefore concluded that *Nostoc* was able to fix nitrogen. From the known difficulty of eliminating contaminating organisms from a mucilaginous envelope such as that with which *Nostoc* is always invested, it seems doubtful whether *Streptothrix* was the only such organism present.

Turning to those investigations which had been carried out on cultures of algae of the purity of which there is little or no doubt, it is found that in no case had the results indicated a fixation of nitrogen. Schramm (23) studied a greater number of species than any earlier investigator, and his biological technique was very carefully worked out. He found that in the absence of combined nitrogen no growth took place with any of the species used, except for an "incipient ephemeral growth" which could only be detected with difficulty, and which he attributed to the unavoidable introduction of traces of combined nitrogen in the inoculum. This inability of the algae to grow in the absence of combined nitrogen, seemed to him to be conclusive evidence that they were unable, under these conditions, to assimilate free nitrogen. This conclusion one would scarcely be disposed to dispute since, in the absence of combined nitrogen, growth and fixation of free nitrogen are mutually interdependent.

Since 1914, papers on the subject have been published by Moore and his co-workers (17,18) and most recently by Wann (25). The former claimed to have shown that fresh water and marine algae could assimilate free nitrogen. Their conclusions are, however, of doubtful validity, since the evidence that their cultures of algae were free from bacteria cannot be regarded as adequate. The elimination of bacteria from an algal culture is a matter of considerable difficulty, necessitating a special technique and taking a considerable time. Moore worked with cultures which he had made no attempt to purify, and based his conclusions as to the absence of bacteria solely on the absence of turbidity of the liquid at the end of eight days. The sheaths of algae growing under natural conditions are invariably inhabited by bacteria which, by reason of their position, do not cause any turbidity in the surrounding fluid; the absence of turbidity is therefore no criterion of the absence of bacteria. Moore, indeed, admits the possibility of the presence of bacteria in his cultures, and his arguments in favour of nitrogen-fixation by the algae rather than by the associated bacteria, if present, are unconvincing in view of the work of Kossowitsch and others.

It may therefore be fairly stated that at the time of publication of

Wann's paper(25) there was no satisfactory evidence of the ability of algae to assimilate free nitrogen, and, indeed, that all the more critical investigations had given results definitely against such a possibility.

Wann's experiments were carried out on very similar lines to those of Schramm(23), but with the important difference that combined nitrogen was intentionally included in all the media, since Schramm had failed to induce growth in its absence. By analysing his cultures at the beginning and at the end of the experiment, Wann obtained results from which he concluded that all the algae tested by him possessed the power of assimilating appreciable quantities of atmospheric nitrogen in the presence of combined nitrogen in the form of nitrate, together with glucose. The cultural technique adopted by Wann did not differ fundamentally from that of Schramm, but owing to the necessity for chemical analysis as a criterion of fixation, certain modifications had to be introduced. These consisted chiefly in the use of an agar medium instead of purified sand, and in the culture of the algae in the Kjeldahl flasks in which the analyses were ultimately to be made. Wann worked with pure cultures of seven species of algae, of which two had been used by Schramm. It is unnecessary to describe his cultural technique in more detail at this point, since that used by the present authors, to be described later, is essentially the same. The chemical methods used by Wann for the determination of the total nitrogen in his cultures are discussed in detail later.

The question of nitrogen-fixation by algae is of considerable interest from the general point of view of plant physiology; it is of still greater interest with reference to soil fertility. Of all the nutrient elements on which plants depend for growth, nitrogen is of the greatest general importance in practice; in this country crop yields are limited probably more by deficiency of nitrogen than by lack of any other single element. Any agency which helps to increase the amount of combined nitrogen in the soil may therefore be an important factor in soil fertility. The regular occurrence of algae in cultivated soils makes it very desirable that Wann's conclusions should be verified. The investigation described in the present paper was therefore undertaken.

II. EXPERIMENTAL METHOD.

A. General.

An attempt was made to reproduce as nearly as possible the conditions which obtained in Wann's experiment, as described in his paper. It was decided, however, to limit the investigation to four species of algae, growing them on six solid media selected from among those used

by Wann and giving, according to him, the most striking results. The cultures were grown in Kjeldahl flasks of 500 c.c. capacity in which they could be finally subjected to chemical analysis: these were numbered consecutively from 1 to 96.

(1) *Cleaning of apparatus.* After careful washing in the ordinary way the Kjeldahl flasks were specially cleaned by projecting into each of them a jet of live steam for at least an hour, after which they were carefully rinsed with freshly distilled nitrogen-free water and dried; in this way the possibility of error by the solution of nitrogenous substances from the surface of the glass was precluded.

Erlenmeyer flasks and boiling tubes were cleaned with a strong solution of potassium dichromate in concentrated sulphuric acid, rinsed with distilled water and inverted in a steamer for three hours, finally being rinsed with nitrogen-free water and dried. Gas washing bottles were treated in the same way except that the steaming was omitted. Calcium chloride tubes were boiled for half an hour in Hudson's soap solution before being steamed. Glass tubing was cleaned successively by means of small wads of cotton-wool saturated with water and methylated spirit. Rubber tubing was fixed in a long piece to a tap and water allowed to run slowly through it for about fifteen hours. It was then cut into lengths of $1\frac{1}{2}$–2 inches suitable for making the connections, and rinsed with distilled nitrogen-free water. Rubber stoppers were scrubbed and then soaked in running water for some hours: they were finally rinsed with distilled nitrogen-free water.

By these means it could be safely concluded that all nitrogenous and toxic substances had been removed from the apparatus before use.

(2) *Assembly of the apparatus.* The apparatus was set up in the laboratory in a well-lighted corner close to south and east windows, on a table covered with white American cloth to provide maximum illumination, and was supported in a specially constructed wooden stand painted white. It consisted of six parallel series of flasks, fitted together by means of bent glass tubing and rubber connections and attached at their extremities to the six side branches of two wide bored tubes, referred to subsequently as the inlet and outlet tubes. (See Plates XXIa and XXII.)

In order to ensure optimum growth of the cultures air was drawn daily through the apparatus by means of a filter pump attached to a central side branch of the outlet tube. It was drawn from outside the building through a long piece of "Compo" tubing attached to a central branch of the inlet tube and was filtered and washed in a special apparatus at the beginning of each series of flasks, as shown in Text-fig. 1a.

After passing through the calcium chloride tube *a*, filled with unsterilised cotton-wool to remove suspended particles, the air was bubbled slowly first through the wash bottle *c* containing 150 c.c. of a 25 per cent. solution of sulphuric acid, to remove traces of ammonia, and then through

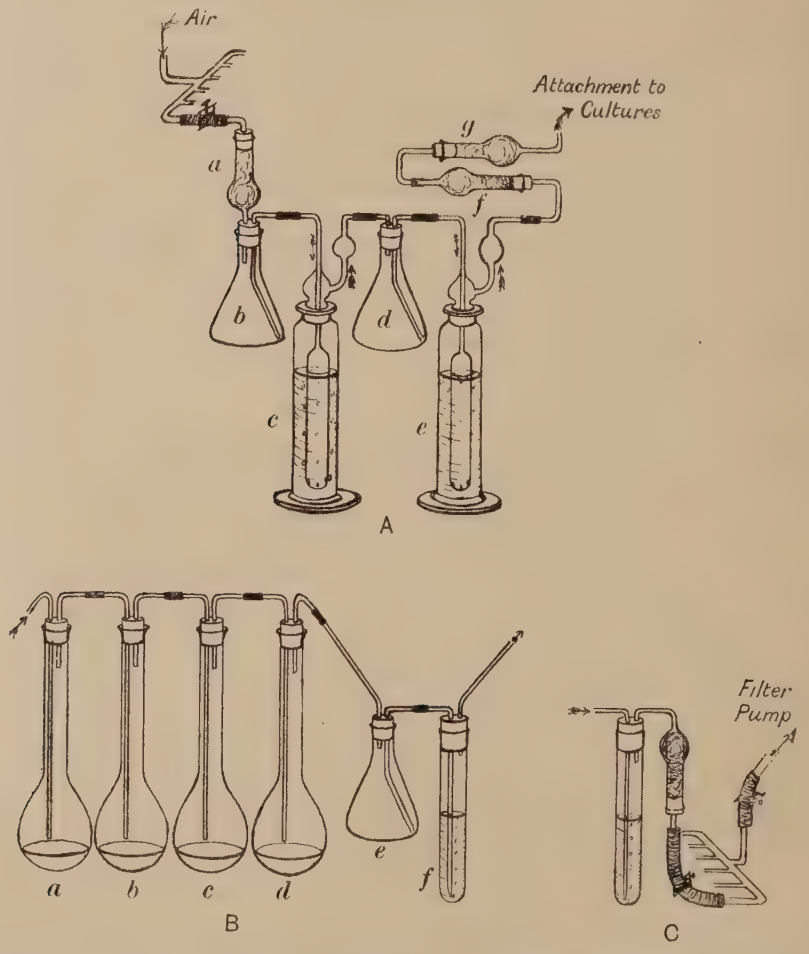

Fig. 1. Diagram showing details of apparatus. A. Apparatus for washing and filtering air, attached to 7-branched inlet tube; *a*, calcium chloride tube packed with cotton-wool; *b* and *d*, safety flasks; *c*, wash bottle containing 25 per cent. sulphuric acid solution; *e*, ditto, containing 1 per cent. sodium bicarbonate solution; *f* and *g*, as *a*, but sterile. B. A single group of sterile flasks (four in each series); *a*, uninoculated control; *b*–*d*, culture flasks; *e*, safety flask; *f*, boiling tube containing *N*/10 sulphuric acid. C. End of series of culture flasks attached to 7-branched outlet tube.

the wash bottle *e* containing 150 c.c. of a 1 per cent. solution of sodium bicarbonate, to remove traces of nitrous gases while still allowing the free passage of atmospheric carbon dioxide into the apparatus. It was finally passed through the two sterile calcium chloride tubes *f* and *g* filled with cotton-wool, and thus entered the series of culture flasks free from traces of nitrogenous compounds and from bacterial and fungal contaminations.

Each of the six series of flasks consisted of sixteen Kjeldahl flasks separated into four groups of four. At the end of each group was placed a boiling tube containing 50 c.c. *N*/10 sulphuric acid free from nitrogen in order to absorb any traces of ammonia that might be liberated from the media during the experiment, together with a safety flask to prevent any sucking back of the acid into the culture flasks. Each flask was provided with a two-holed rubber stopper through which passed two bent glass tubes for the passage of air through the apparatus. In the culture flasks the end of the inlet tube reached nearly to the surface of the medium to ensure that the atmosphere in the flask was changed at each aeration (see Text-fig. 1 B). Each series was terminated by a sterile calcium chloride tube filled with cotton-wool, through which the air passed before leaving the apparatus. This was included to prevent possible contamination of the cultures by the sucking back of air when the filter pump was disconnected. By means of screw clips on the pressure tubing which joined the six series of flasks to the branches of the main inlet and outlet tubes, the rate of passage of air during aeration could be regulated separately for each series, and while aeration was not proceeding each series could be completely closed off from the rest of the apparatus.

The apparatus was first completely assembled empty to ensure that all the connections were as accurately in register as possible. The stoppers fitted with their tubes were then marked, each with the number of the flask to which it belonged. The apparatus was then dismantled, the rubber connections being left attached to the inlet tubes of the flasks. Each stopper with its tubes, the upper ends of which had first been provided with paper caps, was separately wrapped up in glazed paper and the packet numbered. The *N*/10 acid was placed in the acid tubes; these and the safety flasks were then plugged with cotton-wool.

The numbered Kjeldahl flasks were provided with loops of copper wire by which they were suspended on a 2-pan balance and weighed empty. One hundred and fifty cubic centimetres of the appropriate medium were then introduced hot into each, and a cotton-wool plug

inserted in the neck. As soon as the medium was cold the plug was removed for a few moments while the flask and contents were weighed. In this way the exact weight of medium in each flask was determined. Four spare Kjeldahl flasks of each medium were poured for use in the determinations of the initial nitrogen content of the medium and to provide a reserve in case of accidents; they were scattered at random through the series so as to obtain samples of medium as representative as possible of the whole. The small remaining quantities of medium were placed in Erlenmeyer flasks of 70 c.c. capacity for sub-cultures of the organisms to be used in the experiment.

B. Cultural.

(3) *The Media.* Wann concluded that the fixation of atmospheric nitrogen took place only in algal cultures grown on media containing nitrate in the presence of glucose, and his figures indicated a much greater fixation when all the nitrogen was present as nitrate than when, as in the case of ammonium nitrate, half the nitrogen was present as ammonia. There was no clear indication of fixation either on media in which nitrogen was supplied wholly as some compound other than a nitrate, or in the absence of glucose even when nitrate was present.

Consequently, for use in the present experiment, three of Wann's media were selected having as a common basis a non-nitrogenous mineral salts solution devised by Schramm, but differing from one another in the salt added as source of nitrogen. Each of these media was set up in two series, one without added sugar and the other containing 1 per cent. glucose. A stock solution of mineral salts was used for the whole experiment; 25 litres was made up and this sufficed for the whole of the media and for most of the preliminary chemical tests necessary to establish a suitable method of analysis of the media in the flasks.

It contained:

Magnesium sulphate ($MgSO_4$, $7H_2O$)	0·410 gm.	per litre of water distilled in a silver still.
Calcium chloride ($CaCl_2$, $6H_2O$)	0·243 gm.	
·005 per cent. solution of ferrous sulphate ($FeSO_4$, $7H_2O$)	50 c.c.	
Dipotassium hydrogen phosphate (K_2HPO_4)	0·200 gm.	

In preparing this solution it was found necessary to dissolve the potassium phosphate separately in a small quantity of water and to add it in this condition to the solution of the other salts in the rest of the water. In this way only a slight turbidity of the liquid was produced which did not settle and it was possible to obtain a uniform sample of the solution.

Of the stock solution a sufficient volume was taken, 7 litres, to make up the whole of the two media containing any one source of nitrogen;

the appropriate nitrogenous salt was dissolved in this solution which was then divided into two equal parts. To each of these was added 1·5 per cent. of washed agar and the mixture autoclaved and filtered. After filtration, 150 c.c. portions of the hot medium were measured out from one flask into twenty weighed Kjeldahl flasks which were plugged with cotton-wool and left to cool. The filtered medium in the second flask was provided with 1 per cent. glucose and thoroughly shaken before being similarly treated. The six media were thus of the following composition, with the addition of 1·5 per cent. of agar in every case:

	Flasks
I. Stock solution + ·05 % ammonium nitrate [NH_4NO_3]	1–16 + 4 extra
I A. As I + 1 % glucose	17–32 + 4 „
II. Stock solution + ·0942 % ammonium sulphate [$(NH_4)_2SO_4$] ...	33–48 + 4 „
II A. As II + 1 % glucose	49–64 + 4 „
III. Stock solution + ·1475 % calcium nitrate [$Ca(NO_3)_2 4H_2O$] ...	65–80 + 4 „
III A. As III + 1 % glucose	81–96 + 4 „

The amounts of the nitrogenous salts added to the media were calculated so as to give approximately equal quantities of nitrogen in all.

Owing to an unexpected delay in filling the flasks with medium III A, which necessitated its being kept hot on the water bath for some time, a further quantity of water was added to this medium. This accounts for its slightly lower nitrogen content compared with medium III.

(4) *Sterilisation.* After weighing, the flasks of medium were autoclaved at 115° for 15 minutes and placed in order in the stand: when cold, the plugs were covered with caps of tinfoil to prevent evaporation and the flasks left for two weeks before inoculation. This waiting period made it quite certain that none of the flasks contained contaminations before inoculation. The check flasks were analysed on the day on which inoculation of the culture flasks was carried out.

The rubber stoppers and glass tubes and the calcium chloride tubes filled with cotton-wool, all in their numbered paper packets, were autoclaved at 115° for 15 minutes and then dried quickly in the drying room before being stored. The acid tubes and safety flasks were similarly autoclaved and replaced in position in the stand.

(5) *Species of algae taken.* Four species of algae which had previously been obtained in pure culture[1] were selected for investigation. All four

[1] Species A, C and D were obtained in pure culture by one of the authors working by the courtesy of Prof. R. Chodat in his laboratory at the University of Geneva, and under his direction. The grateful thanks of this author are tendered to Prof. Chodat not only for his kind hospitality but also for his invaluable help and encouragement. Species A was isolated from impure material collected at Mount St Bernard. Species C and D from a soil sample taken near Geneva.

Species B was presented in pure culture by Dr M. Denis of Clermont University, France, to whom the grateful acknowledgments of the authors are made. The material was originally obtained from a subaerial habitat.

species were unicellular green algae which for convenience may be designated by the letters A, B, C, and D. Among the lower algae the task of isolation and identification of the various closely related species is one of extreme difficulty, and it would be out of place in the present paper to go into this question in detail. It is hoped, however, shortly to publish an account of the general morphological and physiological characters of the four algae, when a more accurate designation of the species may be possible. The following particulars are sufficient for the present purpose.

Species A, *Chlorella* sp. nova affinis *Chl. coelastroides* Chod., with round or oval cells provided with a chloroplast which completely lines the cell-wall except for a single circular perforation on one side and which contains a conspicuous pyrenoid. Multiplication takes place with the formation of four to eight autospores, or more on sugary media. It differs from *Chl. coelastroides* Chod. chiefly in the form of the colonies on various media.

Species B, *Pleurococcus* sp., a very minute species with oval cells, rarely remaining attached to one another after division. This alga was described as a species of *Stichococcus* (4). It appears, however, to be more like a *Pleurococcus*, in that division has been observed in three planes and a minute pyrenoid is amost invariably present. In old cultures there is always a small proportion of larger cells in which typical protococcoid division has been observed.

Species C, *Cystococcus* sp. In young and vigorously growing cultures this species exists almost entirely in a palmelloid condition with a copious enveloping mucilage; multiplication takes place in this state by successive bipartition, but in no circumstances have zoogonidia been observed. In ageing cultures the cells become enlarged and round themselves off, assuming a typical *Cystococcus* form, in which eventually multiplication may take place by the production of autospores.

Species D, *Scenedesmus* sp. in the sense of Chodat: this designation is given tentatively pending a further investigation of the characters of the alga. It is an ill-defined species which is usually chlorelloid in form, but which in the presence of calcium nitrate and glucose exhibits a distinct tendency to form rather irregular coenobia with the cells slightly pointed and thickened at the two poles. It periodically enters into a resting state with the formation of enlarged thick-walled resting spores containing an orange pigment.

(6) *Inoculation.* Previous observations on these species grown in pure culture had demonstrated that when inoculated for the first time on to a given medium they tend to grow much more slowly and less luxuriantly

than after one or more subsequent reinoculations on to the same medium. Hence preliminary stab cultures of each of the four species were made on each of the media to be used in the experiment. For Species A, B and C sub-cultures of the second generation on each medium were obtained, but for Species D only the first sub-cultures were available. A liquid suspension of each of the cultures was made by transferring the alga by means of a sterile glass spoon from the surface of the agar to a sterile solution of nitrogen-free mineral salts, and well shaking the mixture. Hence the amount of combined nitrogen present in the small quantity of inoculum added to each flask was negligible.

In setting up the experiment, each group of Kjeldahl flasks with its accompanying acid tube and safety flask was removed separately to the inoculation chamber, which had previously been washed down with lysol. Here three of the culture flasks were inoculated each with eight to ten drops of the appropriate algal suspension while the fourth was left uninoculated as a control. Sterile capillary Pasteur pipettes, freshly drawn out, were used for making the inoculations, one for each flask. After inoculation each flask was quickly fitted with its own stopper, freshly removed from its paper wrapping, and the whole group connected together in its final form, aseptic conditions being aimed at throughout. This completed unit was then transferred to its proper position in the laboratory, the paper caps on the open ends of the tubes being left on until the units were connected together in their proper sequence. All the stoppers and rubber connections were immediately coated with melted paraffin wax in order to render all joints completely airtight.

A plan of the experiment is given in Text-fig. 2, which shows the relative positions of the control and culture flasks and of the species and media in respect to one another.

The inoculation of the cultures was carried out on three successive days, Series I and I A on May 3rd, II and II A on May 4th, III and III A on May 5th: air was drawn through the completed apparatus for the first time on the morning of May 6th, 1922.

C. Chemical.

(7) *Methods of Analysis.* Although, from the biological side, Wann's work appears to be adequate, the same cannot be said of the chemical side. It is now necessary to consider this point in detail in order to show in what way the errors in Wann's analytical methods arose, how his conclusions are invalidated by these errors, and how such errors were avoided in the present investigation.

It is well known that the Kjeldahl method is not directly applicable to materials containing more than a very small proportion of their nitrogen in the form of nitrate, owing to the loss of nitric acid which occurs during the oxidation with strong sulphuric acid. Several means have been devised for overcoming this difficulty; the best known consists in a treatment of the substance to be analysed with salicylic acid or phenol dissolved in strong sulphuric acid (6,11). By these means, working under suitable conditions, it is possible to fix the whole of the nitric acid in the form of a nitro compound, which is then reduced to an amino compound by the addition of sodium thiosulphate or zinc dust. After such treatment the digestion with strong sulphuric acid may be carried out in the usual manner until all the organic matter has been oxidised and the nitrogen is all converted to sulphate of ammonia. This method of preventing loss of nitric acid cannot, however, be used in the presence of more than traces of water, for the quantitative conversion of nitric acid to nitro compound can only be effected in concentrated sulphuric acid. The method can therefore be applied to agar media containing nitrate and a large amount of water, such as those in question, only after the removal of the bulk of that water without loss of nitrogen. The method used by Wann for the removal of water may be quoted from his paper: "A considerable part of the water of the medium was boiled off in the presence of 10 c.c. of concentrated sulphuric acid, the process being continued until active foaming began." The boiling down of the medium in the presence of 10 c.c. of strong sulphuric acid until active foaming occurred, that is, until the concentration of the sulphuric acid is high, will necessarily result in the boiling off and loss of a considerable part of the nitric acid set free by the sulphuric acid. The subsequent addition of phenol-sulphuric acid to the concentrated liquid for the purpose of fixing the nitric acid is rather in the nature of "shutting the stable door after the horse has gone."

In the absence of precise information regarding the exact method used for boiling down it is impossible to say how much nitric acid was lost in Wann's analyses. Table I shows the extent of such loss in an experiment carried out by the authors, in which two 150 gm. portions of medium containing nitrate were boiled in a Kjeldahl flask connected to a condenser, after the addition of sulphuric acid. Boiling was continued until active foaming, accompanied by charring, began. The amount of nitrate in the distillate was then determined by reduction to ammonia with Devarda's alloy.

Table I. *Loss of nitric acid caused by boiling down a nitrate medium with the addition of sulphuric acid.*

150 gm. of medium containing mineral salts, agar and 0·2000 gm. pure potassium nitrate.

Nitrate present in medium (0·2000 gm. KNO_3) mgm.	Volume of concentrated H_2SO_4 added c.c.	Nitrogen found in distillate mgm.	Nitrogen boiled off %
27·7	10	3·9	14·1
27·7	1	1·5	5·4

It is seen that in using 10 c.c. of sulphuric acid (Wann's conditions) no less than 14·1 per cent. of the nitric nitrogen originally present has been boiled off and collected in the distillate. Wann no doubt added sulphuric acid in order to prevent the loss of any ammonia from ammonium salts or organic nitrogen compounds, and it was thought possible, though unlikely, that the addition of a much smaller amount of sulphuric acid would eliminate the loss of nitric acid on boiling down. That the addition of only 1 c.c. of sulphuric acid was still fraught with a loss of 5·4 per cent. is seen from the table. The loss observed in the presence of 10 c.c. of sulphuric acid might well be much higher if boiling were continued a little longer, especially if an open flask were used.

The bearing of these results on Wann's conclusions is obvious. Not only do they render untrustworthy his analyses of media containing nitrate, but they also make it likely that the whole of his apparent fixations are illusory.

It must be borne in mind that at the end of the experiment a portion of the nitrogen originally present as nitrate would have been converted by the organisms into organic substances, in which form it would not be driven off on boiling down with sulphuric acid. In the cultures, therefore, there would be a greater amount of nitrogen immune from this loss than in the controls, so that the proportion of the nitrogen originally present which would be recovered would be greater after the growth of algae. The differences obtained when the nitrogen recovered from Wann's controls is subtracted from that recovered from his cultures, instead of being an indication of fixation, as concluded by Wann, may merely indicate that in the analyses more nitrogen had been lost from the controls than from the cultures.

The illusion of fixation is increased by the fact that this difference in recovery of nitrogen from controls and from cultures would be greater the bigger the growth of the algae in the culture, *i.e.* the greater the amount of the original nitrate converted to organic material. This inter-

pretation of Wann's results is strengthened by the fact that he obtained apparent fixation only on those media containing nitrate, that is, only under circumstances when this analytical loss was possible, and that the apparent fixation was greater when all the nitrogen was originally present as nitrate ($Ca(NO_3)_2$) and only roughly half as much when only half the nitrogen was present as nitrate (NH_4NO_3). For all other sources of combined nitrogen there was no evidence of fixation in his results.

It should also be pointed out that whereas the agreement between the results of Wann's parallel check analyses, as reported in his paper, is usually fairly good for those media not containing nitrate, the same cannot be said in the case of media containing nitrate. In the analyses of the latter, differences up to 1·7 mg. of nitrogen per 100 gm. of medium are recorded, and it is reasonable to suppose that the differences were saved from being still greater only by the maintenance of fairly standard conditions during the boiling down of the medium.

The method used by Wann to test the trustworthiness of his method of analysis consisted in the addition of 2 gm. of agar, presumably in the solid condition, to 10 c.c. of ammonium nitrate solution, and the addition thereto of phenol sulphuric acid followed by the usual treatment. This was clearly no check on the method actually used for the media, in that the preliminary boiling down with the addition of sulphuric acid was omitted. Had a true check analysis been carried out, including all the steps used in the actual determinations, the source of error here demonstrated could hardly fail to have been detected.

It is thus possible to explain the whole of the apparent fixation observed by Wann as due to faulty analytical methods; and although it cannot be stated with certainty that there was no fixation in his experiments, it must be pointed out that his methods were not capable of demonstrating it even if it occurred, in the case of media containing nitrate. Moreover, since in the present authors' experiments no evidence of fixation could be found, using methods of analysis which were not subject to the errors inherent in those used by Wann, it is rendered unlikely that any fixation occurred in Wann's experiments.

The method of analysis used by the authors for the determination of total nitrogen in the media containing nitrate was a combination of the Ulsch method(24) with the Kjeldahl method. The nitrate in the media was converted into ammonium sulphate in acid solution, without previous concentration, by treatment with finely divided iron ("reduced iron"). This reaction takes place quantitatively with great readiness. The liquid after such treatment contains the whole of its nitrogen in the form of

ammonium sulphate or organic compounds, and may then be concentrated safely and the organic matter removed by digestion with concentrated sulphuric acid in the usual way. The presence of an appreciable quantity of sulphate of iron in the reduced solution gives rise to certain difficulties. In the digestion, these are due to the separation of crystalline ferric sulphate in the strong sulphuric acid, and in the distillation, to the production of a bulky precipitate of ferric hydroxide on the addition of caustic soda. These difficulties can, however, be readily overcome by careful working. Preliminary experiments were carried out to ascertain the smallest amount of iron needed to effect quantitative reduction of the nitrate in the medium, so as to minimise the inconvenience arising from the above causes. The results of these experiments are given in Table II.

Table II. *Recovery of added nitrate in agar media.*

The medium contained mineral salts, agar, and glucose in the same amounts as the media described on p. 384. To separate 150 c.c. portions of this medium were added known amounts of pure potassium nitrate.

Amount of reduced iron used gm.	Nitrogen added as KNO_3 mgm.	Nitrogen found in 150 gm. of medium alone mgm.	Nitrogen found in 150 gm. of medium $+KNO_3$ mgm.	Nitrate nitrogen recovered mgm.	Nitrate nitrogen recovered %	
2	27·3	4·8	30·5	25·7	94·2	
3	27·3	4·8	31·6	26·8	98·2	
5	27·3	4·8	31·8	27·0	99·0	
5	27·3	4·8	32·1	27·3	100·0	Mean 100·0
10	27·3	4·8	32·4	27·6	101·0	

It is seen that, working with a medium corresponding in composition to that used for the cultures and under conditions otherwise identical with those adopted in the final method (as described below), the recovery of the nitrogen of the added nitrate was incomplete with 2 gm. of reduced iron, almost complete with 3 gm. and complete with 5 gm. or more.

The method finally adopted for all media containing nitrates (*i.e.* media I, I A, III and III A) was therefore as follows: Ten cubic centimetres of 50 per cent. (by vol.) sulphuric acid (nitrogen-free[1]) were added to the medium in the Kjeldahl flask and the whole melted on the water-bath and cooled. Ten cubic centimetres of concentrated sulphuric acid were then added, followed by 5 gm. of reduced iron. The flask was fitted with a rubber stopper through which passed a calcium chloride tube filled with glass beads and coarse quartz fragments, and gently warmed. Heating was so regulated that the liquid came to the boil when the greater

[1] The reagents used were nitrogen-free throughout.

part of the iron had dissolved. After boiling for a few minutes the contents of the calcium chloride tube were washed into the flask, and boiling continued for a few minutes, and the tube again washed into the flask, and removed. The liquid was then boiled down until charring began. Bumping was liable to occur, and the contents of the flask often had to be kept continually shaken to facilitate regular boiling. Twenty-five cubic centimetres of strong sulphuric acid were then added and the mixture digested till frothing ceased; then a crystal of copper sulphate was added and the digestion continued till all organic matter was oxidised and the contents of the flask were light brown in colour. Any black specks in the neck were then washed down with 5 c.c. of strong sulphuric acid and the digestion continued for one hour.

The cooled residue was diluted with water, 110 c.c. of 50 per cent. caustic soda and a few pieces of pure granulated zinc were added, and the liquid was distilled from the Kjeldahl flask with the usual trap, through a block tin condenser, with combustion glass delivery tube. The receiver contained 25 c.c. $N/10$ sulphuric acid, and distillation was continued until at least 125 c.c. of liquid had passed over. The excess of acid was titrated with $N/10$ sodium hydroxide using methyl red as indicator, carbon dioxide being boiled off before the end point was finally determined.

III. EXPERIMENTAL RESULTS.

A. Growth Observations.

(1) *Contaminations.* As was inevitable in an experiment of this extent and complexity, a certain number of contaminations appeared in the culture flasks within a short time of the setting up of the experiment. These were dealt with in one of three ways.

(*a*) The colony of the contaminating organism was cut out with a sterile thin glass spoon, and the agar removed and weighed. This method was found to be unsatisfactory on account of the difficulty of determining the exact limits of fungal colonies, and the following was therefore adopted. (*b*) In all cases where possible, contaminated flasks were removed from the series before the infection could spread to the neighbouring flasks, and their places filled with freshly inoculated spare flasks. This method was adopted for flasks 68–72, 73 and 88. In some flasks, however, a fungal colony grew, the mycelium of which was so diffuse as completely to escape notice until considerable damage had been done, when resort had to be made to a third device. (*c*) Flask 10 was a case of this kind; the only evidence of contamination was a number of minute black dots

appearing on the surface of the agar a month after inoculation. While it was still debatable whether these were actually contaminations, similar structures appeared in flasks 10 and 11, and the whole series was, as a precaution, isolated from the rest of the apparatus by means of plugs of sterile cotton-wool inserted in the glass tubes connecting flasks 9 and 10 and flask 13 and the adjacent acid tube. Contaminations appearing more than seventeen or eighteen days after the setting up of the apparatus were all treated by this method of introducing cotton-wool plugs, since it was considered inadvisable to insert fresh cultures so long after the beginning of the experiment. It proved effective in every case in preventing the spread of the infection.

In one flask, No. 27, the end of the inlet tube almost touched the surface of the agar, and algae grew in the moisture which accumulated on the end of the tube. Owing to a sucking back of the air in the series of flasks this moisture was carried over into the control flask, No. 26, which became secondarily inoculated with Species C.

In consequence of the extended duration of the experiment a few of the rubber connections became somewhat impoverished towards the end of the time, in spite of frequent applications of fresh wax. As a result of the sudden perforation of the connection preceding flask 85 during the process of aeration, air entered the apparatus directly from the laboratory without filtration and caused the contamination of flasks 85–87. At this period the experiment had already been in progress for more than five months, and on the appearance of the contaminating colonies an attempt was made, by immediate analysis of all the flasks of the group, to determine the nitrogen-content of the medium before the growth of the contaminating fungi could essentially affect its composition. The close agreement between the values obtained for all the flasks of the group indicates that this was successful. In flasks 85 and 86 the contaminations grew very rapidly, but in flask 87 there was only a single minute fleck of mycelium whose effect must have been negligible. Similar minute contaminations appeared within four days of the end of the experiment in several other flasks marked in Text-fig. 2 with an asterisk. Except in those cases indicated in the diagram by a large cross, the extent and duration of growth of the contaminating organisms were so small, however, that it is very improbable that they could have affected the main issue of the experiment, and such flasks are therefore included among those giving trustworthy results.

(2) *Growth of the Cultures.* Visible signs of growth appeared in the cultures within four to five days of inoculation on the media containing

glucose and in nine or ten days on the media without glucose. Periodic records were made of the development of the four organisms on the different media and it was observed that the triplicate cultures developed simultaneously and at the same rate. In Text-fig. 2 is indicated diagrammatically the amount of growth at the end of the experiment together with the proportion of the surface of the agar covered with the algae.

For convenience of comparison the cultures have been grouped according to their development into ten classes, as shown in the following table:

Table III.

	Degree of growth	Description of growth: On surface of agar	Description of growth: Between agar and glass	Percentage recovery of nitrogen (average). See pp. 405–6
1	Extremely luxuriant	Continuous stratum several mm. thick over whole of surface	As on surface, but rather less thick	96·72
2	Very luxuriant	Not quite continuous stratum, several mm. thick	Thinner than in 1	97·24
3	Luxuriant	Continuous stratum not more than 1 mm. thick	As in 2	96·77
4	Heavy	Dense skin-like stratum over whole surface, $\frac{1}{4}$–$\frac{1}{2}$ mm. thick	Slight growth	98·86
5	Very good	Similar to 4, but thinner; or rapid initial growth followed by staling of cultures after about six weeks	Very little growth	99·95
6	Good	Fairly rapid initial growth followed by early staling of cultures	No growth	99·55
7	Fairly good	Rather diffuse, thin, continuous stratum	Slight rim of growth	99·11
8	Very fair	Diffuse stratum, not continuous	Very slight growth	99·97
9	Fair	Very thin diffuse stratum, dotted with denser pin-head colonies	No growth	100·20
10	Slight	No definite stratum, but a large number of scattered pin-head colonies	No growth	99·28

In all of these classes except 9 and 10 (Species A on medium II A, Species B and C on medium III and Species D on medium I A) there was a very considerable amount of growth, enough in any case to affect profoundly the medium on which the organism was growing.

The species all attained their maximum development on media containing glucose, Species A and B on that with ammonium nitrate, and

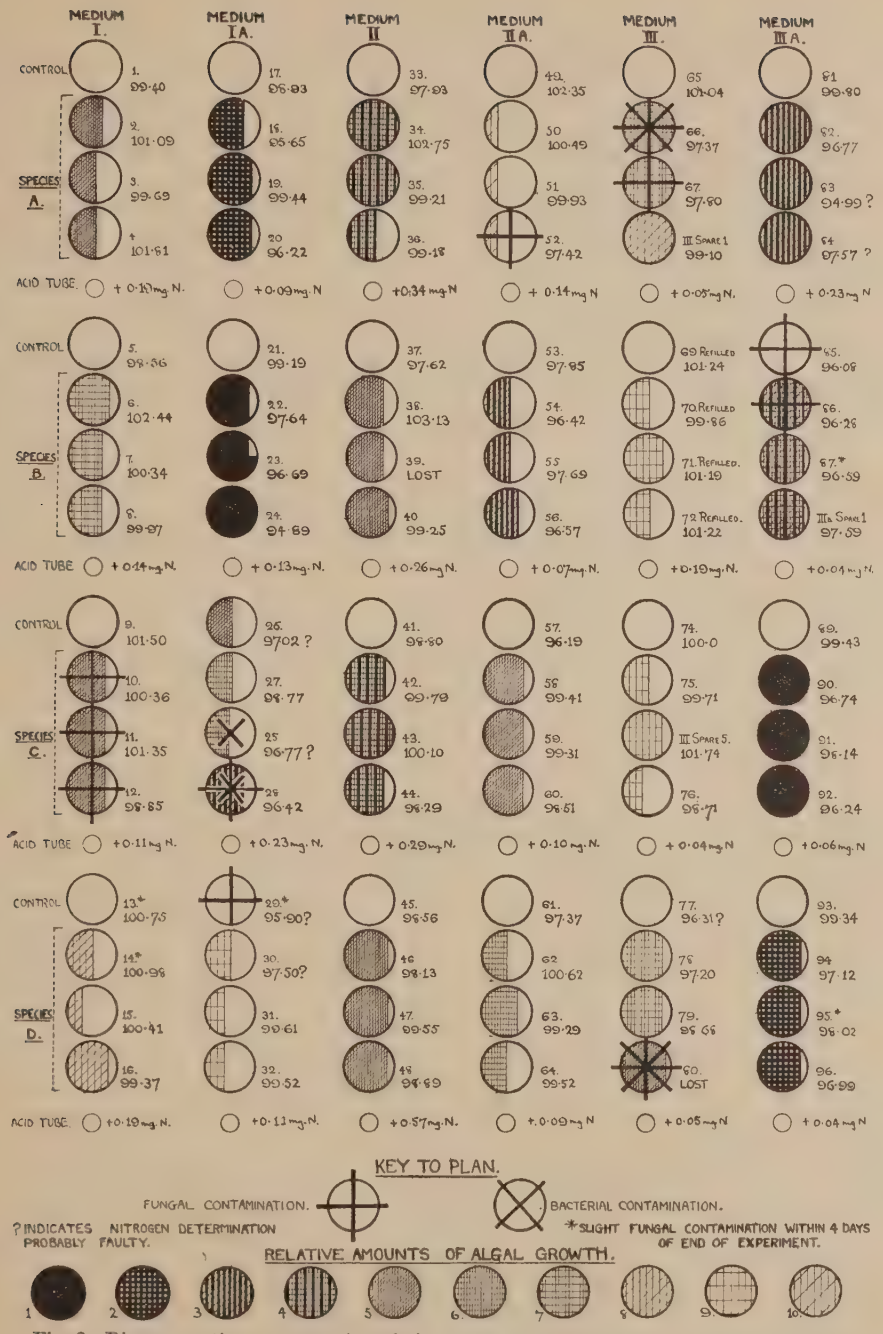

Fig. 2. Diagrammatic representation of the experiment, showing the arrangement of the media and the species and the relative amounts of growth (for explanation of latter see Table III). Of the two numbers against each flask, the upper is the serial number of the flask and the lower indicates the percentage recovery of the nitrogen originally present (for details see pp. 396–9). Similarly the amount of nitrogen found in each acid tube at the end of the experiment is shown.

Species C and D on that with calcium nitrate; in all of these cultures growth continued vigorously to the end of the experiment. Species C gave further conspicuous evidence of its physiological activity in the accumulation of large bubbles of gas, presumably oxygen given out during photosynthesis, between the agar and the glass and within the gelatinous stratum on the surface of the agar.

Species C is further noteworthy in that on media I A and II A, after a rapid initial growth, the stratum gradually lost colour until at the end of three months it had become completely colourless. No further growth took place. This phenomenon has been observed in all cultures of this organism on these media and is probably due to the accumulation of some toxic product.

Species D was conspicuous on medium III A for periodic changes in its colour. Observations on parallel cultures not included in the experiment showed these changes to be due to the periodic formation of thick-walled resting-cells containing an orange pigment. On germination these resting-cells gave rise to green vegetative cells and so caused a regeneration of the green colour of the stratum. There was no bacterial contamination in these cultures as was demonstrated by microscopic examination and by transference to a medium containing peptone and glucose.

Text-fig. 2 brings out very clearly the varying reactions of the four species to the three nitrogenous salts used, both in the presence and in the absence of glucose. Without sugar, the medium containing ammonium sulphate proved the most satisfactory for all four species, while that containing calcium nitrate was least good for three of them, A, B and C. In the presence of sugar, however, the calcium nitrate medium yielded a more luxuriant growth for the whole series than either of the other media; that containing ammonium nitrate was very good for Species A and B but failed to provide the conditions required by Species C and D.

Whether these differences in development are specific reactions of the four algae to the particular compounds supplied as source of nitrogen, or whether they are due to slight differences in the H-ion concentration of the media has not yet been determined.

At the end of the experiment each culture was examined microscopically for contamination before being analysed, and the results of these examinations are recorded in Text-fig. 2.

In almost every case the amount of algal growth in a contaminated flask was greater than that in the uncontaminated flasks of the same group, possibly on account of slightly improved conditions produced in the medium by the action of the contaminating organism.

B. Analytical Results.

At the end of the experiment one complete group of flasks was removed daily, beginning on October 30th, 1922, the remaining flasks of the series being closed off by a screw clip on the rubber connection next to the group removed.

The determination of total nitrogen in each culture and control flask was always begun immediately the microscopic examination of the contents (see above) had been completed. For media I, I A, III and III A the special method described on pp. 391 and 392 was used. For media II and II A the ordinary Kjeldahl method was employed, the precautions during distillation, etc., being the same as those described in the special method just mentioned. Nitrogen was determined in the contents of the acid tubes by the micro-Kjeldahl method, rather than by direct distillation with soda, in case any organic nitrogen compounds should have been passed over into the acid.

The results of the determinations are given in the following six tables, one for each medium. Less trustworthy values are enclosed in square brackets. Reference should be made to Text-fig. 2 for details as to contaminated and doubtful analyses.

Table IV.

Medium I. Nitrogen originally present as ammonium nitrate. No glucose.

Flask No.	Species	Weight of medium, gms.	Initial nitrogen content, mgm.	Final nitrogen content, mgm.	Gain (+) or loss (−) of nitrogen, mgm.	Initial nitrogen per 100 gm. of medium, mgm.	Final nitrogen per 100 gm. of medium, mgm.	Gain (+) or loss (−) of nitrogen per 100 gm. of medium, mgm.	Percentage recovery of nitrogen taken
Check	—	147·75	29·93	—	—	20·25[1]	—	—	—
,,	—	148·04	30·11	—	—	20·34[1]	—	—	—
,,	—	150·25	30·67	—	—	20·41[1]	—	—	—
1	Control	148·30	30·15	29·97	−0·18	20·33	20·21	−0·12	99·40
2	A	149·42	30·38	30·71	+0·33	20·33	20·55	+0·22	101·09
3	A	148·91	30·27	30·18	−0·09	20·33	20·27	−0·06	99·69
4	A	150·05	30·51	31·06	+0·55	20·33	20·69	+0·36	101·81
Acid tube	—	—	—	0·19	+0·19	—	—	—	—
5	Control	147·58	30·00	29·57	−0·43	20·33	20·04	−0·29	98·56
6	B	148·18	30·13	30·86	+0·73	20·33	20·82	+0·49	102·44
7	B	149·17	30·33	30·43	+0·10	20·33	20·40	+0·07	100·34
8	B	149·48	30·39	30·38	−0·01	20·33	20·33	0·00	99·97
Acid tube	—	—	—	0·14	+0·14	—	—	—	—
9	Control	149·29	30·15	30·60	+0·45	20·33	20·49	+0·06	101·50
10	C	148·85	30·26	[30·37]	+0·11	20·33	20·40	+0·07	[100·36]
11	C	147·49	29·99	[30·39]	+0·40	20·33	20·60	+0·27	[101·35]
12	C	148·89	30·27	[29·92]	−0·35	20·33	20·10	−0·23	[98·85]
Acid tube	—	—	—	0·11	+0·11	—	—	—	—
13	Control	147·29	29·94	30·17	+0·23	20·33	20·48	+0·15	100·75
14	D	149·45	30·38	30·68	+0·30	20·33	20·53	+0·20	100·98
15	D	148·67	30·23	30·35	+0·12	20·33	20·41	+0·08	100·41
16	D	149·00	30·29	30·10	−0·19	20·33	20·20	−0·13	99·37
Acid tube	—	—	—	0·19	+0·19	—	—	—	—
	Total	—	483·67	486·37	+2·70	—	—	—	100·6

[1] Mean=20·33.

Table V.

Medium I A. Nitrogen originally present as ammonium nitrate, plus 1 per cent. of glucose.

Flask No.	Species	Weight of medium, gms.	Initial nitrogen content, mgm.	Final nitrogen content, mgm.	Gain (+) or loss (−) of nitrogen, mgm.	Initial nitrogen per 100 gm. of medium, mgm.	Final nitrogen per 100 gm. of medium, mgm.	Gain (+) or loss (−) of nitrogen per 100 gm. of medium, mgm.	Percentage recovery of nitrogen taken
Check	—	147·46	29·83	—	—	20·22[1]	—	—	—
,,	—	149·47	30·45	—	—	20·37[1]	—	—	—
,,	—	146·72	29·87	—	—	20·35[1]	—	—	—
17	Control	149·31	30·33	30·00	−0·33	20·31	20·10	−0·21	98·93
18	A	148·77	30·22	28·90	−1·32	20·31	19·43	−0·88	95·65
19	A	148·19	30·10	29·93	−0·17	20·31	20·20	−0·11	99·44
20	A	149·12	30·29	29·14	−1·15	20·31	19·55	−0·76	96·22
Acid tube	—	—	—	0·09	+0·09	—	—	—	—
21	Control	149·07	30·28	30·03	−0·25	20·31	20·14	−0·17	99·19
22	B	148·20	30·10	29·39	−0·71	20·31	19·83	−0·48	97·64
23	B	149·20	30·30	29·30	−1·00	20·31	19·64	−0·67	96·69
24	B	147·72	30·00	28·47	−1·53	20·31	19·28	−1·03	94·89
Acid tube	—	—	—	0·13	+0·13	—	—	—	—
25	C	148·11	30·08	[29·11]	−0·87	20·31	19·66	−0·65	[96·77]
26	C	148·13	30·09	[29·19]	−0·90	20·31	19·70	−0·61	[97·02]
27	C	146·99	29·85	29·48	−0·37	20·31	20·05	−0·26	98·77
28	Control	148·44	30·15	[29·07]	−1·08	20·31	19·58	−0·73	[96·42]
Acid tube	—	—	—	0·23	+0·23	—	—	—	—
29	Control	147·61	29·98	[28·75]	−1·23	20·31	19·48	−0·83	[95·90]
30	D	146·45	29·74	[29·00]	−0·74	20·31	19·80	−0·51	[97·50]
31	D	148·93	30·25	30·13	−0·12	20·31	20·24	−0·07	99·61
32	D	148·23	30·11	29·96	−0·15	20·31	20·21	−0·10	99·52
Acid tube	—	—	—	0·11	+0·11	—	—	—	—
	Total		481·87	470·41	−11·46	—	—	—	97·7

[1] Mean=20·31.

Table VI.

Medium II. Nitrogen originally present as ammonium sulphate. No glucose.

Check	—	149·12	34·39	—	—	22·99[1]	—	—	—
,,	—	148·34	33·87	—	—	22·83[1]	—	—	—
,,	—	148·89	34·04	—	—	22·85[1]	—	—	—
33	Control	148·47	33·99	33·28	−0·71	22·89	22·41	−0·48	97·93
34	A	148·69	34·04	34·97	+0·93	22·89	23·50	+0·61	102·75
35	A	148·83	33·84	33·57	−0·27	22·89	22·56	−0·33	99·21
36	A	146·60	33·56	33·28	−0·28	22·89	22·70	−0·19	99·18
Acid tube	—	—	—	0·34	+0·34	—	—	—	—
37	Control	148·40	33·97	33·16	−0·81	22·89	22·33	−0·56	97·62
38	B	148·43	33·98	35·04	+1·06	22·89	23·60	+0·71	103·13
39	B	—	—	lost	—	—	—	—	—
40	B	147·11	33·67	33·42	−0·25	22·89	22·72	−0·17	99·25
Acid tube	—	—	—	0·26	+0·26	—	—	—	—
41	Control	148·39	33·97	33·56	−0·41	22·89	22·61	−0·28	98·80
42	C	147·89	33·85	33·78	−0·07	22·89	22·84	−0·05	99·79
43	C	147·78	33·83	33·86	+0·03	22·89	22·91	+0·02	100·10
44	C	148·23	33·93	33·35	−0·58	22·89	22·50	−0·39	98·29
Acid tube	—	—	—	0·29	+0·29	—	—	—	—
45	Control	146·63	33·56	33·08	−0·48	22·89	22·57	−0·32	98·56
46	D	147·80	33·83	33·20	−0·63	22·89	22·47	−0·42	98·13
47	D	148·20	33·92	33·77	−0·15	22·89	22·79	−0·10	99·55
48	D	148·84	34·07	33·70	−0·37	22·89	22·65	−0·24	98·89
Acid tube	—	—	—	0·57	+0·57	—	—	—	—
	Total	—	508·01	506·48	−1·53	—	—	—	99·7

[1] Mean=22·89.

Table VII.

Medium II A. Nitrogen originally present as ammonium sulphate, plus 1 per cent. of glucose.

Flask No.	Species	Weight of medium, gms.	Initial nitrogen content, mgm.	Final nitrogen content, mgm.	Gain (+) or loss (−) of nitrogen, mgm.	Initial nitrogen per 100 gm. of medium, mgm.	Final nitrogen per 100 gm. of medium, mgm.	Gain (+) or loss (−) of nitrogen per 100 gm. of medium, mgm.	Percentage recovery of nitrogen taken
Check	—	149·59	34·05	—	—	22·76[1]	—	—	—
,,	—	148·99	34·00	—	—	22·82[1]	—	—	—
,,	—	149·43	34·05	—	—	22·80[1]	—	—	—
49	Control	149·19	34·00	34·80	+0·80	22·79	23·33	+0·54	102·35
50	A	149·34	34·04	34·20	+0·16	22·79	22·90	−0·11	100·49
51	A	149·25	34·01	33·99	−0·02	22·79	22·78	−0·01	99·93
52	A	147·73	33·67	[32·80]	−0·87	22·79	22·20	−0·59	[97·42]
Acid tube	—	—	—	0·14	+0·14	—	—	—	—
53	Control	149·01	33·96	33·23	−0·73	22·79	22·31	−0·48	97·85
54	B	147·77	33·68	32·47	−1·21	22·79	21·97	−0·82	96·42
55	B	147·51	33·62	32·84	−0·78	22·79	22·26	−0·53	97·69
56	B	148·61	33·88	32·72	−1·16	22·79	22·01	−0·78	96·57
Acid tube	—	—	—	0·07	+0·07	—	—	—	—
57	Control	148·72	33·89	32·60	−1·29	22·79	21·93	−0·86	96·19
58	C	148·58	33·86	33·66	−0·20	22·79	22·65	−0·14	99·41
59	C	149·52	34·08	33·84	−0·24	22·79	22·63	−0·16	99·31
60	C	149·58	34·09	33·58	−0·51	22·79	22·46	−0·33	98·51
Acid tube	—	—	—	0·10	+0·10	—	—	—	—
61	Control	149·61	34·10	33·20	−0·90	22·79	22·19	−0·60	97·37
62	D	150·02	34·19	34·40	+0·21	22·79	22·92	+0·13	100·62
63	D	149·16	33·99	33·75	−0·24	22·79	22·63	−0·16	99·29
64	D	149·34	34·04	33·87	−0·17	22·79	22·68	−0·11	99·52
Acid tube	—	—	—	0·09	+0·09	—	—	—	—
	Total	—	543·10	536·35	−6·75	—	—	—	98·8

[1] Mean=22·79.

Table VIII.

Medium III. Nitrogen originally present as calcium nitrate. No glucose.

Check	—	147·90	30·74	—	—	20·78[1]	—	—	—
,,	—	148·66	30·70	—	—	20·64[1]	—	—	—
,,	—	148·13	30·81	—	—	20·80[1]	—	—	—
Check (Second batch of medium)	—	149·24	31·35	—	—	21·00[2]	—	—	—
Check (Second batch of medium)	—	149·46	31·40	—	—	21·10[2]	—	—	—
65	Control	149·22	30·95	31·27	+0·32	20·74	20·97	+0·23	101·04
66	A	148·31	30·76	[29·95]	−0·81	20·74	20·20	−0·54	[97·37]
67	A	148·64	30·83	[30·15]	−0·68	20·74	20·29	−0·45	[97·80]
Spare No. 1	A	148·89	30·88	30·60	−0·28	20·74	20·55	−0·19	99·10
Acid tube	—	—	—	0·05	+0·05	—	—	—	—
69 (refilled with second batch of medium)	Control	149·03	31·31	31·70	+0·39	21·01	21·27	+0·26	101·24
70 (refilled with second batch of medium)	B	148·91	31·29	31·24	−0·05	21·01	20·98	−0·03	99·86
71 (refilled with second batch of medium)	B	149·20	31·35	31·72	+0·37	21·01	21·26	+0·25	101·19
72 (refilled with second batch of medium)	B	148·12	31·12	31·50	+0·38	21·01	21·27	+0·26	101·22
Acid tube	—	—	—	0·19	+0·19	—	—	—	—
74	Control	149·49	31·00	31·00	0·00	20·74	20·74	0·00	100·00
75 (Spare No. 5 (Second batch of medium))	C	147·58	30·61	30·52	−0·09	20·74	20·68	−0·06	99·71
76 (Spare No. 5 (Second batch of medium))	C	148·63	31·23	31·77	+0·54	21·01	21·38	+0·37	101·74
Acid tube	—	148·49	30·80	30·40	−0·40	20·74	20·47	−0·27	98·71
77	Control	148·93	30·89	[29·75]	−1·14	20·74	20·00	−0·74	[96·31]
78	D	148·81	30·86	30·00	−0·86	20·74	20·16	−0·58	97·20
79	D	148·04	30·70	30·30	−0·40	20·74	20·46	−0·28	98·68
80	D	—	—	lost	—	—	—	—	—
Acid tube	—	—	—	0·05	+0·05	—	—	—	—
	Total	—	464·58	462·20	−2·38	—	—	—	99·5

[1] Mean=20·74. [2] Mean=21·01.

Table IX.

Medium III A. Nitrogen originally present as calcium nitrate; plus 1 per cent. of glucose.

Flask No.	Species	Weight of medium, gms.	Initial nitrogen content, mgm.	Final nitrogen content, mgm.	Gain (+) or loss (−) of nitrogen, mgm.	Initial nitrogen per 100 gm. of medium, mgm.	Final nitrogen per 100 gm. of medium, mgm.	Gain (+) or loss (−) of nitrogen per 100 gm. of medium, mgm.	Percentage recovery of nitrogen taken
Check	—	148·28	29·17	—	—	19·66[1]	—	—	—
,,	—	148·68	29·59	—	—	19·89[1]	—	—	—
,,	—	148·26	29·13	—	—	19·63[1]	—	—	—
81	Control	148·81	29·36	29·30	−0·06	19·73	19·70	−0·03	99·80
82	A	148·49	29·30	28·35	−0·95	19·73	19·20	−0·53	96·77
83	A	148·07	29·22	[27·75]	−1·47	19·73	18·73	−1·00	[94·99]
84	A	148·58	29·32	[28·60]	−0·72	19·73	19·25	−0·48	[97·57]
Acid tube	—	—	—	0·23	+0·23	—	—	—	—
85	Control	147·97	29·20	[28·05]	−1·15	19·73	18·95	−0·78	[96·08]
86	B	148·46	29·29	[28·20]	−1·09	19·73	19·00	−0·73	[96·28]
87	B	148·50	29·30	28·30	−1·00	19·73	19·05	−0·68	96·59
Spare No. 1	B	146·99	29·00	28·30	−0·70	19·73	19·25	−0·48	97·59
Acid tube	—	—	—	0·04	+0·04	—	—	—	—
89	Control	147·82	29·17	29·00	−0·17	19·73	19·63	−0·10	99·43
90	C	148·54	29·31	28·35	−0·96	19·73	19·10	−0·63	96·74
91	C	147·71	29·14	28·60	−0·54	19·73	19·37	−0·36	98·14
92	C	148·51	29·30	28·20	−1·10	19·73	18·98	−0·75	96·24
Acid tube	—	—	—	0·06	+0·06	—	—	—	—
93	Control	148·47	29·29	29·10	−0·19	19·73	19·60	−0·13	99·34
94	D	146·12	28·83	28·00	−0·83	19·73	19·15	−0·58	97·12
95	D	146·60	28·92	28·35	−0·57	19·73	19·35	−0·38	98·02
96	D	148·41	29·28	28·40	−0·88	19·73	19·15	−0·58	96·99
Acid tube	—	—	—	0·04	+0·04	—	—	—	—
	Total	—	467·23	455·22	−12·01	—	—	—	97·4

[1] Mean=19·73.

IV. DISCUSSION OF RESULTS.

A careful examination of the above analytical results reveals the fact that in only 21 cases out of a total of 94[1] is the figure for the amount of nitrogen recovered greater than that for the nitrogen originally in the medium; moreover, this excess is in all cases less than 0·75 mg. per 100 gm. of medium, and in all cases but three less than 0·5 mg. In a protracted and complicated experiment of this kind, it is almost impossible to estimate accurately the probable experimental error[2]. From the analytical figures given on p. 391, it is seen that in three determinations on the same medium carried out almost simultaneously the extreme difference between two determinations is 0·6 mg. for 150 gm. of medium, *i.e.* 0·4 mg. per 100 gm. of medium. Similarly, the triplicate check analyses on the media actually used (see Tables IV–IX) exhibit extreme variations up to 0·26 mg. N per 100 gm. of medium. In the case of a difference between two determinations carried out at an interval of several months, during which the flasks containing the media had been subjected to the

[1] Two analyses were lost through accidents.

[2] It is, however, possible to test the agreement between the results of analyses of the contents of inoculated and control flasks with some accuracy, as shown later.

various operations in the experiment, the probable error would certainly be greater than that of three parallel determinations made together[1].

The largest gains of nitrogen found are so little greater than this probable error that the present authors regard them as not significant. In support of this it may be pointed out that:

(1) The flasks which yielded these apparent gains were scattered throughout the whole apparatus.

(2) The apparent gains were obtained for control flasks as well as for inoculated ones.

(3) Against these apparent gains must be reckoned a greater number of apparent losses.

(4) There is no correlation between the amount of growth in the flasks and the apparent gains; indeed, the most luxuriant cultures gave apparent losses in all cases (see below).

The detailed analytical results in Tables IV–IX are summarised in Table X, which gives the mean percentage recovery of the nitrogen originally taken, for each set, for each medium as a whole, for each species as a whole, and for the whole experiment, the control flasks being averaged separately from the cultures. Certain of the figures in Tables IV–X are known to be less trustworthy than the rest owing to contaminations or the probability of errors in the analyses. Such figures are enclosed in square brackets, and in Table X, where accompanied by a second figure not in square brackets, the latter figure has been obtained by the omission from the calculation of all dubious values.

Taking the experiment as a whole, and excluding doubtful cases, it will be seen that the figures for the mean percentage recovery from controls and from cultures are practically identical (99·25 and 99·28). Moreover, the mean value for each medium, and especially for each species, is only slightly divergent from these figures. The mean values for the separate groups are somewhat more variable but not conspicuously so. The same is true if all the values, including doubtful cases, are taken, the omission of the latter having little influence on the figure obtained. There is thus not the slightest evidence of fixation in these results. That the whole of the differences obtained (with the possible exception of those which, as shown below, may indicate a loss of nitrogen) are probably entirely due to experimental error is indicated by Text-fig. 3. In this figure are four distribution curves, constructed from the values for the percentage recovery of nitrogen in the separate flasks. Curves *A* and *B* have been derived from the trustworthy values for controls and for

[1] See footnote on p. 404.

Table X. *Summary of analytical results.*

Species	Nitrogen		Medium I mgm.	I Recovery %	Medium I A mgm.	I A Recovery %	Medium II mgm.	II Recovery %	Medium II A mgm.	II A Recovery %	Medium III mgm.	III Recovery %	Medium III A mgm.	III A Recovery %	Total for species mgm.	Total for species Recovery %
A	In Control	Taken	30·15	99·4	30·33	98·9	33·99	97·9	34·00	102·4	30·95	101·0	29·36	99·8	188·78	99·9
		Found	29·97		30·00		33·28		34·80		31·27		29·30		188·62	
	In Cultures	Taken	91·16	101·1	90·61	97·2	101·44	100·7	[101·72]	[99·4]	[92·47]	[98·1]	[87·84]	[96·7]	[565·24]	[98·9]
		Found[1]	92·14		88·06		102·16		68·05 [101·13] 68·33	100·4	30·88 [90·75] 30·65	99·3	29·30 [84·93] 28·35	96·8	411·44 [559·17] 409·69	99·6
B	In Control	Taken	30·00	98·6	30·28	99·2	33·97	97·6	33·96	97·9	31·31	101·2	29·20	[96·1]	[188·72]	[98·4]
		Found	29·57		30·03		33·16		33·23		31·70		[28·05]		159·52 [185·74] 157·69	98·9
	In Cultures	Taken	90·85	101·1	90·40	96·6	67·65[2]	101·6	101·18	97·0	93·76	101·0	87·59	[96·9]	[531·43]	[98·9]
		Found[1]	91·81		87·29		68·72[2]		98·10		94·65		[84·84]		443·84 [525·45] 440·57	99·3
C	In Control	Taken	30·15	101·5	30·15	[96·4]	33·97	98·8	33·89	96·2	31·00	100·0	29·17	99·4	[188·33]	[98·7]
		Found	30·60		[29·07]		33·56		32·60		31·00		29·00		158·18 [185·83] 156·76	99·1
	In Cultures	Taken	90·52	[100·3]	[90·02]	[97·8]	101·61	99·7	102·03	99·2	92·64	100·1	87·75	97·1	[564·57]	[99·0]
		Found[1]	[90·79]		29·85 [88·01] 29·71	99·5	101·28		101·18		92·73		85·21		413·88 [559·10] 410·11	99·1
D	In Control	Taken	29·94	100·8	29·98	[95·9]	33·56	98·6	34·10	97·4	30·89	[96·3]	29·29	99·3	[187·76]	[98·0]
		Found	30·17		[28·75]		33·08		33·20		[29·75]		29·10		126·89 [184·05] 125·55	98·9
	In Cultures	Taken	90·90	100·5	[90·10]	[99·0]	101·82	99·4	102·22	99·9	61·56[2]	98·0	87·03	97·4	[533·63]	[99·1]
		Found[1]	91·32		60·36 [89·20] 60·20	99·7	101·24		102·11		60·35[2]		84·79		503·89 [529·01] 500·01	99·2
Total for medium															Grand Total	
	In Control	Taken	120·24	100·1	[120·74]	[97·6]	135·49	98·2	135·95	98·4	[124·15]	[99·7]	[117·02]	[98·7]	[753·59]	[98·8]
		Found	120·31		60·61 [117·85] 60·03	99·0	133·08		133·83		93·26 [123·72] 93·97	100·8	87·82 [115·45] 87·40	99·5	633·37 [744·24] 628·62	**99·25**
	In Cultures	Taken	[363·43] 272·91	[100·7]	[361·13]	[97·6]	372·52	100·2	[407·15]	[98·8]	[340·43]	[99·4]	[350·21]	[97·0]	[2194·97]	[99·0]
		Found[1]	[366·06 275·27	100·9	271·22 [352·56] 265·26	97·8	373·40		373·48 [402·46] 369·66	99·0	278·84 [338·48] 278·38	99·8	204·08 [339·77] 198·35	97·2	1773·05 [2172·73] 1760·38	**99·28**

N.B. Less trustworthy values are enclosed in square brackets. See p. 401; also Text-fig. 2 for details.

[1] Including acid tubes.

[2] Two cultures only.

cultures respectively; curve *C* is a combination of curves *A* and *B*, while curve *D* contains in addition those less trustworthy values excluded from curve *C*. It will be seen that within the limits of the available data, the curves are all of the normal type, with a modal value of 98·5–99·5. In other words, the mean value for the percentage recovery of nitrogen, taking the experiment as a whole (99·25–99·28, see Table X), has a real meaning.

A statistical analysis of the data from which these curves were constructed[1] gives the following results. The 20 control values in curve *A* are scattered with a standard deviation of 1·55 per cent., that of the

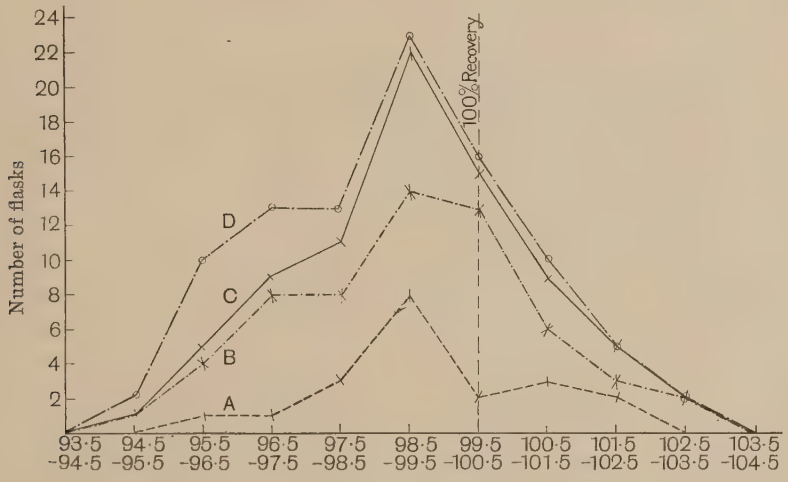

Fig. 3. Curves showing distribution of values obtained for percentage recovery of nitrogen. For description see text.

59 values from inoculated flasks in curve *B* being 1·82 per cent.; the difference between these values is not significant, and together they give an average of 1·76 per cent. From this value the standard error of the difference is calculated from the formula $1{\cdot}76\sqrt{\frac{1}{20}+\frac{1}{59}}=0{\cdot}455$. The mean of the control values is 99·30, while that of the values from inoculated flasks is 99·03, a slightly lower value; the difference, $0{\cdot}27 \pm 0{\cdot}45$, is definitely insignificant, although it is apparent that the method is sufficiently accurate to detect with certainty a difference of only 1 per cent. It should be pointed out that the above mean of the values from inoculated flasks, 99·03, is slightly lower than the percentage recovery

[1] We are indebted to Mr R. A. Fisher, Chief Statistician at this Station, for this analysis.

calculated from the total of all trustworthy values, 99·28 (Table X), largely owing to the inclusion in the latter total of the nitrogen recovered from the acid tubes.

That the values for the percentage recovery of nitrogen are practically the same for controls and for cultures is considered to be the strongest evidence that no fixation has occurred. The fact that the values are slightly less than 100 can only indicate that in the analyses carried out at the end of the experiment the conditions used were not quite identical with those used for the check analyses six months or more earlier, although there was no obvious difference in any detail[1]. It is difficult to suppose that a real loss of nitrogen from the medium in the control flasks could have occurred.

The smallest percentage recoveries were obtained with Species A on medium I A and medium III A, with Species B on medium I A and medium II A and with Species C and D on medium III A. It is interesting to note (1) that these are the cultures in which growth was most luxuriant, although, had fixation occurred, they are those in which one would have expected it to be most marked; (2) that the medium in each case contained sugar; and (3) that in five of the six cases nitrogen was present in the form of nitrate. This might be taken to indicate that on media containing sugar vigorous algal growth results in a loss of gaseous nitrogen. The distribution of the values for the percentage recovery of nitrogen when the cultures are classified according to the amount of algal growth lends some support to this view. The averages of the percentage values for the cultures in each class are shown in Table III; and it is conspicuous that the averages for classes I–III (96·72, 97·24, 96·77) are considerably below the mean value for the control flasks (99·25) while the averages for classes IV–X (98·86, 99·95, 99·55, 99·11, 99·97, 100·20, 99·28) are grouped

[1] Attention has been called by "Student" (*Biometrika*, 1917, XI, 415) to the possibility of a secular error in a series of determinations not all carried out on the same date. He says: "After considerable experience I have not encountered any determination which is not influenced by the date on which it is made, from this it follows that a number of determinations of the same thing made on the same day are likely to lie more closely together than if the repetitions had been made on different days." It is quite possible that the probable error of the difference between the nitrogen-content of the flasks at the end of the experiment and that of the check flasks analysed more than six months earlier is influenced by such a secular error. Some idea of the probable magnitude of such error is given by the result of the analysis of a spare flask of medium III which had remained untouched until October, 1923, *i.e.* 17 months in all. The value for the nitrogen-content of this flask was 21·16 mgm. nitrogen per 100 gm. of medium, compared with the value of 21·01 mgm. found at the beginning of the experiment, a difference of 0·15 mgm. This would appear to indicate that the secular error due to the interval elapsing between the check analyses and the final analyses six months later is almost negligibly small.

fairly regularly though slightly above this mean. When all the flasks of classes I–III are grouped separately from the rest, their percentage values are seen (Table XI, column 1) to have a fairly normal distribution about a modal value of 96·66–97·0 per cent. The flasks of classes IV–X, on the other hand, are distributed about a modal value of 99·33–99·66 (Table XI, column 2) with a range entirely comparable to that of the control flasks (column 3). The means of the values in columns 1 and 2 are 96·93 and 99·61 respectively. The difference between these, 2·65 ± 0·5, is thus significant and appears to indicate that there was a small but definite loss of nitrogen from the luxuriant cultures.

Table XI. *Distribution of percentage values for recovery of nitrogen in relation to amount of algal growth.*

	Numbers of flasks		
Percentage values	(1) Luxuriant growth, Classes I–III. (See Table III)	(2) Heavy to slight growth, Classes IV–X. (See Table III)	(3) No growth, Controls
94·66– 95·00	1	.	.
95·00– 95·33	.	.	.
95·33– 95·66	1	.	.
95·66– 96·00	.	.	.
96·00– 96·33	11	1	1
96·33– 96·66	111	1	.
96·66– 97·00	1111	.	.
97·00– 97·33	1	1	.
97·33– 97·66	1	111	11
97·66– 98·00	1	1	11
98·00– 98·33	11	11	.
98·33– 98·66	.	1	11
98·66– 99·00	.	11111	11
99·00– 99·33	.	111111	1
99·33– 99·66	1	111111	111
99·66–100·00	.	111111	1½
100·00–100·33	.	1	½
100·33–100·66	.	11111	.
100·66–101·00	.	1	1
101·00–101·33	.	111	11
101·33–101·66	.	1	1
101·66–102·00	.	11	.
102·00–102·33	.	.	.
102·33–102·66	.	1	1
102·66–103·00	.	1	.
103·00–103·33	.	1	.

In this connection it should be noted that Wann records a similar though much greater loss with one species of *Chlorella* when nitrogen was present as ammonium nitrate or calcium nitrate. The present authors, however, are not prepared, on the basis of the comparatively limited data available to regard the existence of a denitrifying action of this type as definitely established[1].

V. SUMMARY AND CONCLUSIONS.

The work described in the present paper was undertaken in order to test the results obtained by Wann, on the strength of which this author claimed that green algae were capable, in the presence of nitrate and glucose, of assimilating free nitrogen from the atmosphere.

The present authors working with four species of green algae in pure culture, under conditions as nearly as possible identical with those used by Wann, have entirely failed to corroborate his results, although the amount of growth obtained in the cultures was in almost all cases considerable, and in some luxuriant. The average percentage recovery of nitrogen for the whole experiment was for the cultures 99·28 per cent., and for the controls 99·25 per cent.

Moreover, the method of chemical analysis used by Wann for media containing nitrates is shown to be entirely unreliable, and likely to yield figures which would give the appearance of fixation even when none had occurred. Since it was only on media containing nitrate, and thus liable to give faulty results, that Wann claimed to have obtained definite fixation of nitrogen, his results must be regarded as entirely inconclusive.

The chemical methods used by the present authors were free from the serious sources of error in those used by Wann. While it is quite conceivable that green algae might assimilate atmospheric nitrogen under certain conditions as yet unknown, there is so far no trustworthy evidence that they can do so.

[1] A negative correlation between intensity of growth of algae and percentage recovery of nitrogen would be caused if the complex nitrogen compounds built up by the algae were not entirely broken down to sulphate of ammonia by the digestion with sulphuric acid in the Kjeldahl process. This possibility was investigated by carrying out determinations of nitrogen in dried algal material by the Kjeldahl method and by the absolute (Dumas) method. The percentages of nitrogen found were: Kjeldahl method 3·52 per cent., Dumas method 3·56 per cent. These results appear to rule out the above possibility, the difference between them being less than the experimental error of the determinations on the small amount of material available for analysis. These determinations were kindly carried out by Mr G. C. Sawyer.

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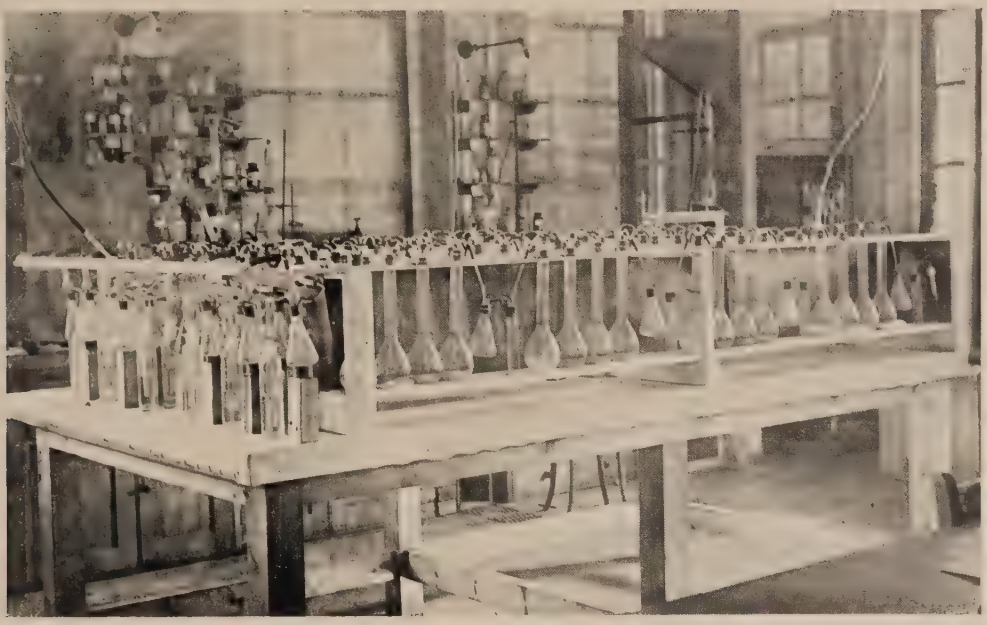
EXPLANATION OF PLATES XXI AND XXII

Plate XXI A. General view of the complete apparatus, showing the air washing and filtering apparatus and the flasks of the first series.

Plate XXI B. A single unit of the experiment (Species D on Medium III A). Note the luxuriant growth in the three inoculated flasks.

Plate XXII. End view of the apparatus, showing the six series of flasks and their connections with the outlet tube.

(*Received July 30th*, 1923.)



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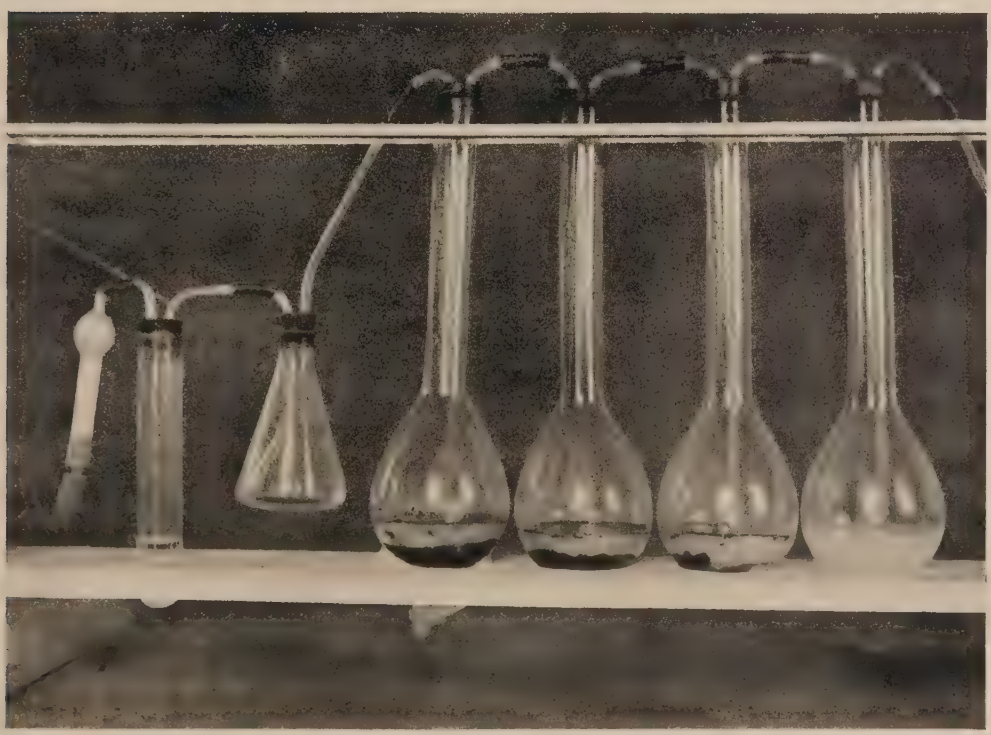

B

BRISTOL AND PAGE.—A Critical Enquiry into the Alleged Fixation of Nitrogen by Green Algae. (pp. 378—408.)

BRISTOL AND PAGE.—A Critical Enquiry into the Alleged Fixation of Nitrogen by Green Algae. (pp. 378—408.)

ON THE LARVAL AND PUPAL STAGES OF *FORCIPOMYIA PICEUS* WINN.[1]

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(With 14 Text-figures.)

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Introduction.

The following investigation was commenced at Birmingham University in 1921 under the supervision of Prof. F. W. Gamble, F.R.S., and was continued at Rothamsted under the direction of Dr A. D. Imms. To

[1] A grant in aid of publication has been received for this communication.

Professor Gamble and to Dr Imms I am indebted for assistance in many ways during the course of this work. For the identification of the imagines I have to thank Mr F. W. Edwards.

The larvae were collected from beneath the bark of dead Conifers (*Larix europaea*) in Sutton Park, Birmingham, and were easily reared upon pieces of bark kept in a moist chamber. Until pupation they were reared in darkness, but were examined each day and the pupae transferred to shallow glass vessels in which they could be observed more easily. The light did not appear to affect them adversely, though under natural conditions they always pupate beneath the bark. The pupal period is from eight to ten days under laboratory conditions. In the laboratory pupation occurred towards the end of February, the imagines emerging during the early part of March. As pupae and empty pupal skins were found at approximately the same times in the open, the artificial laboratory conditions do not appear to affect the times of metamorphosis very greatly.

The larvae to be sectioned were fixed in either Gilson's or Bouin's fluid and embedded in wax with a melting point of 54° C. All sections were cut at 8μ.

External Anatomy of the Larva.

The larva (Text-fig. 1) consists of a well-defined head which is not capable of invagination within the prothorax, and twelve clearly marked body segments, the first and last of which bear ventrally the prothoracic and anal pseudopods respectively. The segments gradually diminish in size posteriorly.

The Head (Text-fig. 2) is a somewhat pear-shaped body bearing on its narrow ventral end the slit-like mouth. It is attached to the first body segment by a flexible lightly chitinized membrane, similar to the membrane joining adjacent segments of the body. The head capsule is formed of smooth firm chitin and bears the eyes, antennae and mouth parts and a number of prominent setae. The antennae (*a*) are situated dorso-laterally in the region where the dorsal and anterior surfaces meet. Each is two-jointed, consisting of a short and broad obtusely conical base from whose apex arises the second joint. The second joint is rod-like and tapers to a blunt apex bearing a minute stiff peg. The eyes (*E*, Text-figs. 1 and 2) are small, circular, and very convex protuberances of transparent chitin.

The vertex of the head bears near its posterior margin a pair of long curved setae projecting dorsally and anteriorly (*p.s.*). Their bases are

separated by about two-thirds of the width of the head. They bend first slightly towards each other and then strongly outwards, so that their apices are separated by considerably more than the width of the head. The antennae arise anteriorly and slightly laterally to the bases of these setae. Anterior to the antennae and more medianly situated, is a second

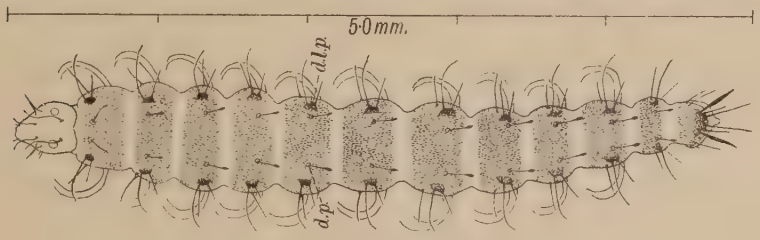

Fig. 1. Dorsal view of Larva.

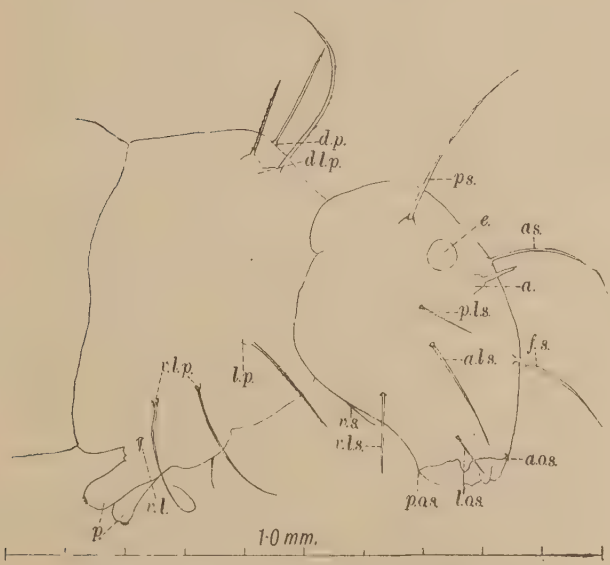

Fig. 2. Lateral view of Head and Prothorax.

pair of dorsal setae (*a.s.*). These project forwards and then curve downwards and outwards. Midway between these and the anterior border of the mouth, the anterior surface of the head bears a pair of frontal setae which diverge from their bases and curve downwards (*f.s.*). The head bears two setae on each side, one of which is much shorter than the other. The short seta is situated postero-ventrally to the antenna, projects

forwards and outwards and is very slightly curved (*p.l.s.*). The longer seta (*a.l.s.*) arises antero-ventrally to the antenna, projects outwards and forwards and is also slightly curved. Ventro-laterally, midway between the mouth and the posterior border of the head, there is on each side a seta projecting downwards and outwards (*v.l.s.*). Slightly posterior to these, two small setae (*v.s.*) project downwards and forwards from the ventral surface. There are six short setae round the mouth; a pre-oral pair (*a.o.s.*) borne on the labrum, a lateral oral pair (*l.o.s.*), and a post-oral pair (*p.o.s.*) borne by the labium.

The Body consists of three thoracic and nine abdominal segments. Segments II to X are, except as regards size and the arrangement of the minute ventral setae which will be considered later, practically identical in external form. Each segment is broadest in the middle and decreases rather abruptly in diameter towards each end. Except for the inter-segmental regions and a narrow strip along the midventral region, the surface is covered with minute dark brown chitinous hooks. The dorsal ones point mainly backwards, the lateral ones mainly downwards.

Dorso-laterally each segment bears an oval papilla of dark brown chitin with an anterior and a posterior apex (*d.l.p.*). In the young larvae each of these papillae is at the summit of a large conical projection of the body wall, but in mature larvae the body wall becomes distended, chiefly by the increase in volume of the fat body, and these projections become practically obliterated. This is the condition shown in Text-fig. 1. Each papilla bears two setae, one on each apex. The anterior seta is strongly curved, projecting first upwards, outwards and slightly forwards and then bent strongly backwards. Under a low magnification it appears to be quite smooth, but with a high magnification it is found to have a few minute projections on its anterior surface. The posterior seta is straight, much shorter than the anterior one and bears numerous sharp projections pointing towards its apex (Text-fig. 2).

Dorsally each segment bears two small papillae of dark brown chitin (*d.p.*) from each of which a single straight and rather short seta projects upwards and backwards making an angle of about 45° with the surface of the segment. The apex of the seta is expanded into a small spear-shaped head which, in the living larva, always bears a small sphere of clear liquid. On each side of the segment is a small conical papilla (Text-fig. 2, *l.p.*) bearing a single seta. This seta is almost straight and has projections similar to those on the posterior dorso-lateral setae. Ventro-laterally the segment bears, on each side, two very small papillae situated close together (Text-fig. 2, *v.l.p.*) and each bearing a long and

delicate curved seta. The basal parts of these setae are straight and project downwards and outwards, and are formed of denser chitin than their apical regions. Their apical two-thirds are bent strongly forwards and then upwards towards the body.

The chief peculiarity of the first body segment is its possession of a prominent ventral pseudopod (Text-fig. 2, *p.*). This is a large, freely movable appendage, more or less conical in form, arising by a broad base from the ventral region of the segment and having its distal end deeply cleft into right and left apices. Each apex is flattened and has a sucker-like appearance; each bears, close to its median border, a small circle of about six strongly chitinized simple hooks. The dorso-lateral papillae are smaller than those borne by the other segments, and the setae borne by the dorsal papillae have no spear-shaped head, are slightly curved and do not bear the drops of liquid mentioned above.

In segment XI each dorso-lateral papilla has only one apex and consequently bears only one seta which, from its size and shape, obviously corresponds to the anterior setae of the dorso-lateral papillae on other segments. There is only one ventro-lateral seta and this is short, only slightly curved, and projects outwards and backwards.

Segment XII differs considerably from any of the other segments. Postero-dorsally it bears a single large oval papilla, the long axis of which is transverse to the long axis of the body. This bears six small conical protuberances each bearing a single seta, all of which are practically straight. The setae are arranged in two transverse rows, four in the anterior row and two in the posterior row. The two median anterior ones project upwards and backwards, diverging somewhat from each other. The two lateral anterior setae project outwards and backwards, rising only very slightly from the horizontal; they are dorso-ventrally flattened. The two posterior setae project backwards and outwards. Two backwardly-projecting setae arise on each side from small papillae on the mid-lateral region of the segment, the more dorsal and posterior of the two setae being the longer.

Postero-ventrally the twelfth segment bears a prominent anal pseudopod on the surface of which is the anal opening (Text-fig. 3). The posterior edge of the pseudopod is produced into a median, backwardly projecting, conical lobe (*m.l.*) which bounds the anus (*an.*) posteriorly. On each side of the anus are two ventrally projecting conical lobes (*l.l.*). The anterior border of the pseudopod is in the form of a rounded semicircular rim which bears a number of prominent, dark brown, chitinous hooks with their apices pointing anteriorly. They are more or less arranged in two

parallel semicircular series. Their shape is somewhat similar to that of a thorn on a briar stem; a curved hook arises from the centre of a basal bar which is firmly attached to the semicircular rim.

The minute ventral setae must be considered separately for thoracic and abdominal segments. The abdominal segments each bear six ventral setae, three on each side. In the first seven segments their arrangement is identical. Two are situated in the anterior half of the segment, one on each side of and close to the median line. In the posterior half of the segment there are two on each side rather far from the median line, the more laterally placed being also the more anteriorly placed of the two. In the eighth abdominal segment the anterior and posterior sets of setae are approximated so that they almost form a single transverse series near the middle of the segment. In the last abdominal segment the setae

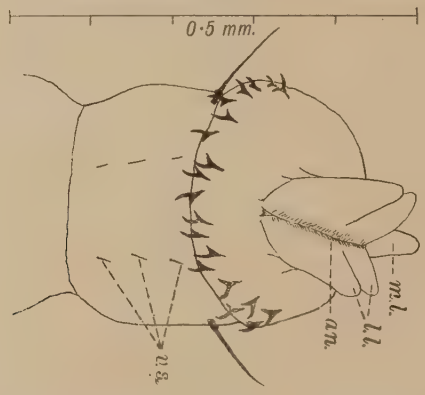

Fig. 3. Ventral view of last body segment of Larva.

are more medianly situated than in the other segments so that they form two longitudinal rows, each of three setae, one on each side of and close to the median line (Text-fig. 3, *v.s.*).

Each thoracic segment bears on each side, near the lateral border of the ventral surface and in the anterior half of the segment, a single minute seta. In the case of the prothoracic segment these are situated on the anterior border of the pseudopod near its base. A short distance posterior to each of these single setae is a group of three very small and slender setae with their bases very close together. In the prothoracic segment they lie just lateral to the base of the pseudopod (Text-fig. 2, *v.l.*). In each group one of the setae is considerably shorter than the other two. These groups of three setae are undoubtedly the structures interpreted by Keilin (10) as vestigeal legs, which he believes to be present,

with very few exceptions, in all Dipterous larvae. Their relation to the imaginal discs of the legs will be considered when dealing with these structures.

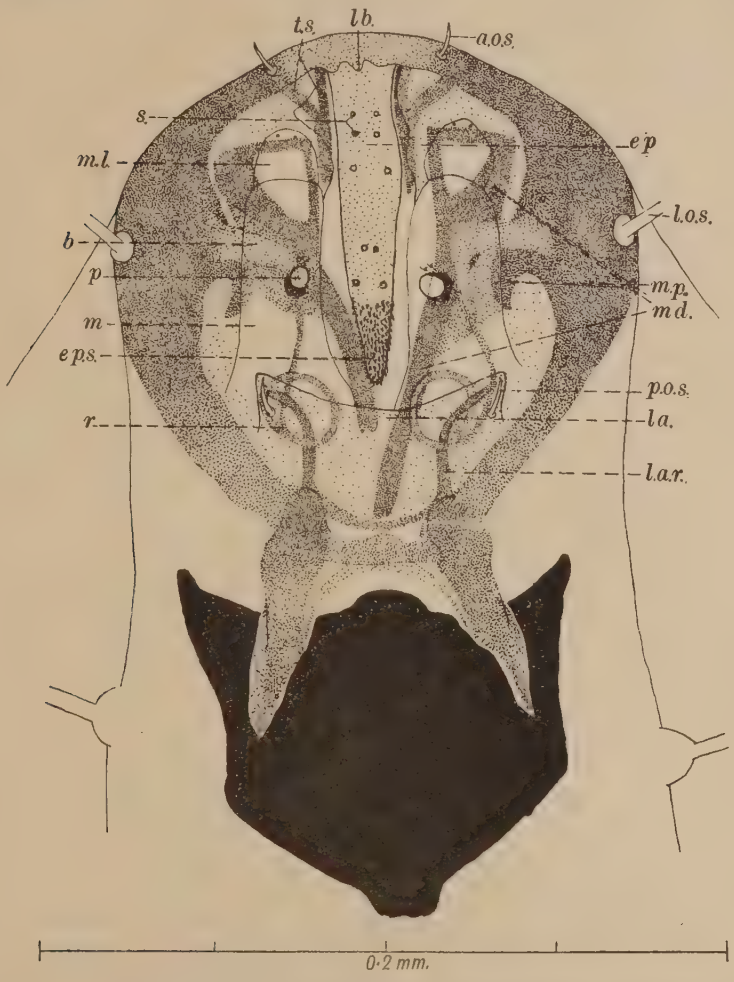

Fig. 4. Ventral view of Larval Mouth Parts. (From a specimen mounted in Canada Balsam.)

The Mouth (Text-fig. 4) is a narrow longitudinal slit on the narrow ventral end of the head. The Labrum (*lb.*) is a small plate overhanging the anterior end of the mouth and bearing the pre-oral setae (*a.o.s.*) on its dorsal surface. The Labium (*la.*) is a somewhat similar plate bordering

the mouth posteriorly and bearing the two post-oral setae (*p.o.s.*) on its posterior or ventral surface. Its free edge is slightly concave, but does not show the conspicuous lobing of the true Chironomid larvae (16). A sagittal section of the head shows that the labium consists of an outer and an inner plate, both heavily chitinized, the free edge of the labium being the point of junction of the two plates. Posteriorly the outer plate is continuous with the ventral chitin of the head, the inner plate with the chitinous intima of the ventral wall of the pharynx. The labium is supported by two curved chitinous rods (*l.a.r.*) whose relations will be described later.

The Maxillae (*m.*) are very rudimentary. Each is a rounded swelling running along the lateral border of the mouth opening and continuous along its whole length with the lateral chitin of the head on one side and the chitin of the pharynx on the other. Its anterior end is differentiated as a rounded lobe (*ml.*) which appears to bear a few minute sense organs whose precise nature I have been unable to determine. Midway along its ventral surface it bears a prominent projection (*p.*) supported by a short hollow cylinder of firm chitin. On the anterior surface of this projection there are two small papillae each bearing an exceedingly minute seta which is probably sensory. The ventral projection is probably to be regarded as the maxillary Palp.

The Epipharynx (*ep.*) is a fold of the dorsal chitin of the mouth cavity, the anterior end of the fold being continuous with the inner surface of the labrum. The ventral surface of the fold lies along the mouth opening which it almost completely fills, its posterior end almost reaching the labium. It thus divides the anterior region of the mouth cavity into right and left halves. The posterior region of the epipharynx, both on its ventral surface and on the ventral regions of the lateral walls, is beset with numerous minute spines (*ep.s.*). The sides of the epipharynx, which are attached dorsally to the roof of the mouth, are thin and longitudinally wrinkled and so are capable of extension. Examination of a living larva shows that the epipharynx can be protruded from the mouth and performs sweeping movements backwards and forwards, its anterior end acting as a hinge. There is little doubt that by these movements the larva sweeps into its mouth the particles of decaying wood and bark upon which it feeds; the spines on its posterior end doubtless increase its efficiency in this respect. In serial sections I have indeed occasionally found such particles adhering to these spines. On the anterior region of its ventral surface the epipharynx bears five pairs of small sense organs (*s*). Sections of this region show that these are relatively deep but narrow cylindrical

pits whose walls are formed of thin smooth chitin. It may reasonably be supposed that they are gustatory in function.

The Mandibles (*md.*) are two stout rods lying parallel to the long axis of the mouth opening through which no part of them is capable of protrusion. Their anterior ends are expanded and articulate with certain regions of the head skeleton which are described below. Their posterior ends are divided into three stout blunt teeth. They do not work against each other but against the inner plate of the labium, and they are only capable of motion through a small arc of a circle in the sagittal plane. They are not themselves capable of approximation to the labium which is normally slightly separated from their apices and consequently there has to be a mechanism, which is described below, whereby the labium is forced forwards against their apices. The strong retractor muscles of the mandibles arise from the postero-lateral regions of the head and converge in a fan-like manner to be inserted into a projection about half-way along the dorsal border of each mandible. The protractor mandibular muscles arise from the antero-lateral regions of the head capsule (anterior to the antennal bases) and converge towards the anterior articular end of each mandible. The tendon passes over the base of the mandible and is inserted into its ventral surface a short distance from its anterior end. The contraction of the retractor muscles draws the mandibular apices dorsally, *i.e.* into the mouth cavity. The protractor muscles have of course the reverse effect. The mandibles are capable of independent motion. Thus in Text-fig. 4 one mandible is in the retracted position while the other is protracted.

I am not aware of any previously described Orthorrhaphous larva whose mandibles move in the sagittal plane. In many (Becker, 1910) the mandibular apices work against the labium and against each other, but their direction of motion is oblique. In view of the fact that the mandibular apices in *Forcipomyia piceus* are incapable of protrusion through the mouth opening and therefore incapable of any prehensile or puncturing action, it is interesting to note that the larva of *Ceratopogon* (= *Forcipomyia*) *Laboulbene* is, according to Perris (21), occasionally predaceous and must therefore have rather differently constructed mouth parts. Perris does not, however, give a detailed description of the mouth parts, and it is not possible to state what the differences are.

The Head Skeleton (Text-figs. 4 and 5) consists of two regions, one being a special development of the chitin surrounding the mouth, the other situated near the centre of the head and being a special development of the chitinous intima of the pharynx. I am unable at present to

give a full account of the structure of the latter region. The two parts are connected ventrally by chitinous rods.

The general shape of the pharyngeal skeleton is that of a V whose base is much thickened and is traversed by the pharyngeal cavity. The apices of the arms of the V lie just beneath the dorsal wall of the head slightly anterior to the antennal bases. Its base is connected by chitinous rods to the labium. Powerful muscles arise from the posterior dorso-lateral regions of the head capsule and are inserted into the posterior

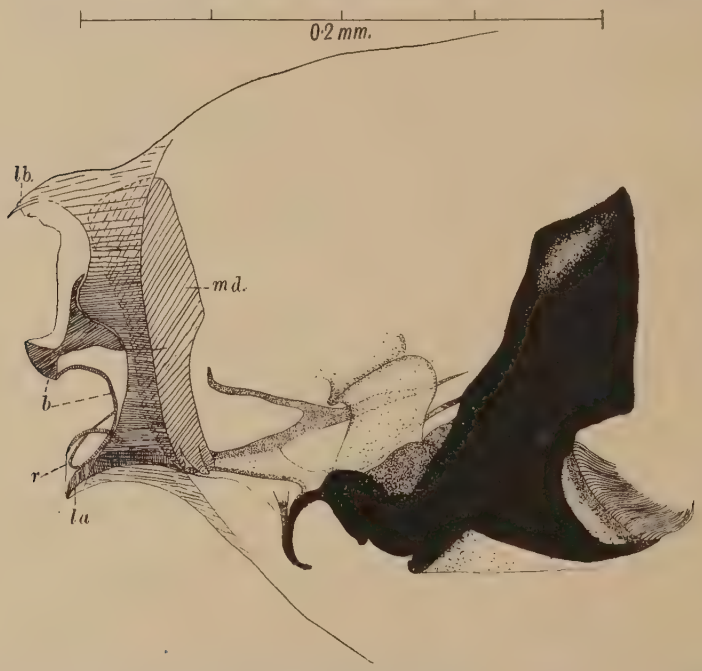

Fig. 5. Lateral view of Larval Mouth Parts and Tentorium. (Somewhat diagrammatic.) (From a potash cleared mount.)

surfaces of the arms of the V near their bases. The contraction of these muscles pulls the pharyngeal skeleton and the attached labium upwards. The antagonistic muscles arise from the anterior dorso-lateral regions of the head capsule and are inserted into the anterior surfaces of the arms of the V, near their bases.

Surrounding the mouth there is a chitinous ring which is very thick and hard except anteriorly. From the thin anterior region of the ring arise two triradiate sclerites (*t.s.*), one on each side. The two anterior arms of each of these sclerites are firmly attached to the ring, while the

third arm projects horizontally backwards into the mouth. Slightly more posteriorly the ring gives off on each side a prominent mesially directed process (*mp.*) and the mandibles articulate between these and the posterior arms of the triradiate sclerites. Close to the base of each lateral process arises a long curved rod with a stout base which projects downwards and slightly mesially (*b.*). The main part of the rod is directed posteriorly at right angles to its base and ends posteriorly just in front of the labium, in a rather large ring (*r.*). The details of its course can be seen in Text-figs. 4 and 5. The curved rods supporting the labium have already been mentioned. Each passes through one of the rings described above, and the purpose of this arrangement is, I believe, as follows. When the labium is drawn upwards by the upward movement of the pharyngeal skeleton, the chitinous rings press against the posterior surfaces of the curved rods supporting the labium and these rods are curved in such a way that this pressure forces them, and the labium with them, forwards against the mandibular apices. This is, however, probably a secondary function only, and there are probably definite muscles for the movement of the labium towards the mandibular apices. The primary function of the chitinous rod and ring is probably to hold the labium in position against the pressure of the mandibles during mastication, and the curve of the rods bearing the ring probably allows a certain amount of play so that the labium is not held absolutely rigidly fixed.

External Anatomy of the Pupa (Text-figs. 6 and 7).

The pupa emerges through a dorsal slit in the larval skin a short distance behind the head, but the posterior end of the pupal abdomen is never withdrawn from the larval skin. During the emergence of the pupa the prominent hooks on the anal pseudopod of the larva act as organs of fixation being firmly attached to the surface of the bark. They again perform this function at the time of emergence of the imago. The pupa drawn in Text-figs. 6 and 7 has been freed from the larval skin. The head, thoracic and abdominal regions are easily distinguished; it will be convenient to deal first with the second of these.

The Thorax is very convex dorsally and is produced posteriorly into a hollow, conical scutellar process (*s.p.*) which projects backwards over the first abdominal segment, and bears a pair of small rounded prominences, one on each side, about half-way along its dorsal surface. The scutellum of the imago occupies the anterior region of this hollow process, and in old pupae the scutellar bristles of the imago can be seen projecting into its posterior prolongation. A median ridge (*r.*), which is interrupted

about half-way along its length by a small flattened area, extends from the anterior border about half-way along the dorsal surface of the thorax. This ridge is formed of two narrow strands of hard chitin, one along each side, the median part of the ridge being much thinner and less densely chitinized. The splitting of the pupal integument for the escape of the imago takes place along the thin apex of this ridge. There are eight slightly curved dorsal spines (*d.s.*) whose arrangement is sufficiently shown in

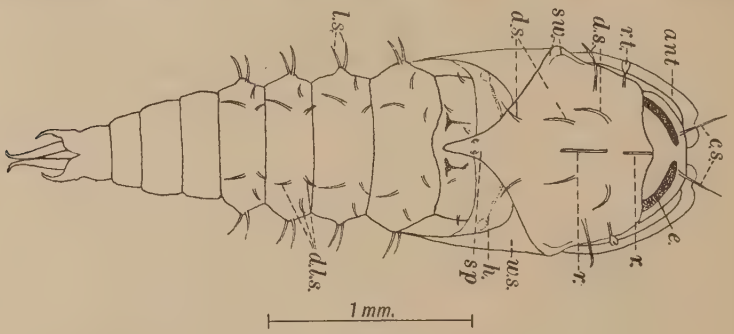

Fig. 6. Dorsal view of ♂ Pupa.

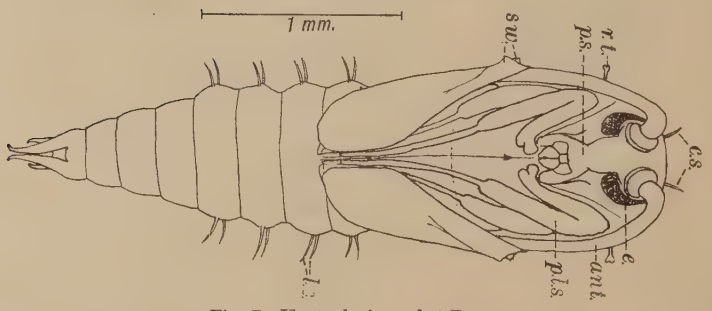

Fig. 7. Ventral view of ♂ Pupa.

Text-fig. 6. Antero-laterally the thorax bears on each side a short knobbed respiratory trumpet (*r.t.*).

The wing sheaths (*w.s.*) arise by a broad base from the lateral region of the thorax just anterior to the base of the scutellar process. Slightly dorsal to the base of each, the lateral wall of the thorax bears two rather prominent swellings, a dorsal and a ventral, situated close together (*sw.*). Beneath these swellings are developed the chitinous sclerites which take part in the articulation of the wing. The wing sheaths are directed posteriorly and curve round on to the ventral surface, being closely applied to the body wall. Their tips extend back to the anterior ventral region

of the third abdominal segment. Their ventral edges lie very close together and between them are the distal ends of the leg sheaths. All but the coxal region of the prothoracic leg sheaths (*p.l.s.*) are clearly visible in a ventral view; only the tip of the coxal sheath can be seen. The disposition of the various parts is shown in Text-fig. 7. Their tips extend almost to the apex of the wing sheaths between whose ventral edges the tarsal region of the prothoracic leg sheaths lies. Only small parts of the meso- and metathoracic leg sheaths are visible, being for the most part covered by either the prothoracic leg sheaths or the wing sheaths. The tips of all of them can be seen between the ventral edges of the wing sheaths. There is no pupal sheath for the haltere though this can be seen clearly below the pupal integument (Text-fig. 6, *h.*). It base lies just below the antero-lateral border of the scutellar process. The shaft passes outwards and backwards and the head lies just above the dorsal border of the wing sheath.

The pupal *Head* region lies antero-ventrally to the thorax and is produced into a proboscis sheath (*p.s.*) which lies close against the antero-ventral region of the thorax, between the prothoracic femoral sheaths. The head bears two short and curved dorsal spines (*c.s.*). From its antero-lateral regions arise the antennal sheaths (*ant.*) which bend back along the lateral borders of the head and thorax, their distal ends being covered by the wing sheaths. In male pupae the base of the sheath is considerably more swollen than in female pupae, corresponding to the greater development of Johnston's organ in the male imagines. The eyes of the imago (*e.*) can be seen beneath the pupal integument occupying the sides of the head just posterior to the bases of the antennae. In old pupae they become very prominent owing to their dense black colour.

Shortly before the emergence of the imagine the sheaths of the various appendages separate slightly from each other and from the surface of the pupa, thus facilitating the withdrawal of the appendages of the imagine.

The pupal *Abdomen* is conical in shape, narrowing rapidly posteriorly, and consists of nine clearly defined segments. The last three, and the posterior two-thirds of the sixth never become freed from the larval skin, and it is probably owing to this that they possess none of the prominent spines which are a conspicuous feature of the first five abdominal segments. These abdominal spines are of the same type as the thoracic ones and are arranged in a dorso-lateral and a lateral series on each side of the body. On segments II to V they are identical in arrangement; each segment bears on each side a pair of lateral spines at about the mid-point

of its lateral surface, the two being close together, one slightly dorsal to the other (*l.s.*); dorso-laterally are three spines on each side whose arrangement is shown in Text-fig. 6 (*dl.s.*); the posterior spine of each group is the largest. The lateral regions of the first abdominal segment being covered by the wing sheaths no lateral spines are developed. Dorso-lateral spines are present but are smaller than those on the other segments and somewhat differently arranged (Text-fig. 6). In male pupae the last abdominal segment is produced backwards into four processes, two larger ventral and two smaller dorsal ones (Text-figs. 6 and 7). In the female pupae only the ventral processes are present. The pupal chitin bears numerous small blunt papillae, considerably smaller than the hooks borne by the larval chitin. The pupal spines are hollow protuberances of the pupal chitin and are not connected to the pupal chitin by a joint. Each lies over a group of somewhat enlarged hypoderm cells each of which sends a protoplasmic prolongation into the cavity of the spine. They thus agree in all respects with Comstock's definition of a "spine"(5) and differ very greatly from the larval "setae." They have an irregularly ringed appearance as though made up of several joints. Under a high magnification this appearance is seen to be due to a number of minute projections on the seta arranged in an irregularly annular manner.

The Respiratory Trumpets consist of a short hollow cylindrical shaft and a distal knob. A trachea runs up the centre of the shaft and opens to the exterior by a small aperture at the apex of the knob. In the shaft the trachea is surrounded by a thin cellular layer which becomes many cells thick in the knob whose cavity it almost fills. The trachea does not show any spiral thickening but its intima is raised up into numerous spines. These are particularly prominent in the part of the trachea lying in the knob and probably act as an occluding apparatus.

Internal Anatomy of the Larva.

The Gut is a cylindrical and practically straight tube, consisting of fore-gut, mid-gut and hind-gut. The Pharynx, which is the anterior region of the fore-gut, extends through the anterior half of the head and opens ventrally at the mouth. I am at present unable to give any account of its structure, but I believe that the complicated sclerites formed from the chitinous intima have a triturating and a straining function.

The Oesophagus is a straight tube of uniform diameter proceeding directly backwards from the posterior end of the pharynx. It traverses the ganglionic mass in the first thoracic segment and ends in the Cardiac Valve in the second thoracic segment. Internally it is lined by a chitinous

intima which is produced into irregular folds. The epithelium is thin and is only distinct (in sections) in the bases of the folds of the intima. The muscular coat is several fibres in thickness and consists exclusively of circular muscles.

Text-fig. 8 is a slightly diagrammatic representation of a sagittal section through the cardiac valve. The oesophagus is continued unchanged down the centre of the valve, at the posterior end of which its epithelium (*e.* and *e'.*) and chitinous intima (*i.* and *i'.*) are reflected outwards and forwards to the anterior end of the valve. Here (*a.*) the reflected layer of epithelium turns posteriorly to become continuous with the epithelium of the cardiac region of the mid-gut (*c.*). The muscular layer of the oesophagus (*m.*) is continued down the valve to its posterior end but is not

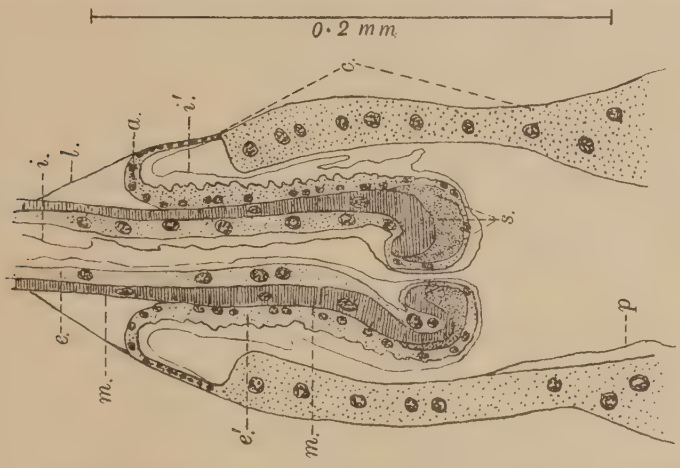

Fig. 8. Sagittal Section of the Larval Cardiac Valve. (Slightly diagrammatic.)

reflected forwards again as is the epithelium. The reflected chitinous intima is continuous over the whole extent of the reflected epithelium from which, however, it is widely separated except at the point where the reflected epithelium becomes continuous with the cardiac epithelium; at this point the chitinous intima ends. Slender ligaments (*l.*) pass from the wall of the oesophagus to the point where the reflected epithelium turns posteriorly.

In *Chironomus* and *Simulia* according to Miall and Hammond (18) and in *Anopheles* according to Imms (8) there is a fairly extensive blood sinus between the two layers of the cardiac valve. In *Forcipomyia* the two layers are in complete apposition except posteriorly; the posterior end of the valve is somewhat swollen and between the two layers there is a

space (*s.*) which I regard as a blood sinus. Compared with the corresponding sinus in the three types mentioned above it is greatly reduced in size, and I am unable to find any communication between it and the general body cavity of the larva.

The reflected epithelium, just in front of its point of junction with the cardiac epithelium, is composed of exceedingly small cells so that the point of junction of the two epithelia is very distinct. A study of late larval stages shows that these small cells are imaginal cells which form the posterior region, and possibly the whole of the imaginal oesophageal epithelium. A ring of imaginal cells in this position is of common and possibly universal occurrence in Dipterous larvae. It is recorded for instance in *Musca* by Kowalevsky (12) and van Rees (24) and in *Chironomus*, *Anthomyia*, *Stratiomys*, *Tanypus* and *Gastrophilus* by Vaney (23). A similar ring occurs in many other holometabolous larvae, *e.g.* in *Formica rufa* according to Pérez (20), and in *Hyponomeuta padella* according to Mme Hufnagel (7).

The cells of the cardiac epithelium are essentially similar to those of the mid-gut epithelium generally except that they never give off masses of secretion (see below) and are non-vacuolated except in late larval stages. There are no muscle fibres in the wall of the cardia.

Posteriorly the cardia becomes continuous with the stomach which is a straight cylindrical tube extending back to the anterior region of the eighth body segment where it joins the hind-gut. It is lined internally throughout its length by a cylindrical Peretrophic Membrane (Text-fig. 10, *p.*). Its wall (Text-fig. 10) consists of a large celled epithelium (*e.*), an inner layer of circular muscles one fibre thick (*c.m.*), an outer layer of isolated longitudinal fibres (*l.m.*) and an external sheathing membrane. The peretrophic membrane is a delicate tube of uniform diameter throughout. In the cardia (Fig. 8) it lies close against the surface of the epithelium from which it can hardly be distinguished unless it is broken away in cutting the sections (*p.*). The diameter of the mid-gut cavity is in most places greater than that of the cardiac cavity, and as the diameter of the peretrophic membrane is constant throughout and equal to the diameter of the cardia, it is in most places widely separated from the epithelium of the mid-gut. For this reason I am inclined to agree with the generally accepted view that the peretrophic membrane is produced by the cells of the cardia. According to Imms (8) this is probably its mode of origin in *Anopheles* but in *Chironomus* it appears, according to Miall and Hammond (18), to be derived from the epithelium of the stomach "either by secretion or conversion."

The epithelium of the stomach consists of large, and for the most part columnar cells with prominent nuclei. Their bases are usually conspicuously vacuolated. Between the internal faces of the cells and the peritrophic membrane, in the middle two-thirds of the stomach, there is usually a granular mass with a slight affinity for plasma stains such as Lichtgrün and Eosin (Fig. 10, *g.s.*). That this mass is secreted by the gut cells may be very clearly seen in almost every transverse section of the region where the secretion occurs. The inner ends of the cells are normally somewhat conical in shape and are formed of densely staining non-vacuolated protoplasm. Certain of them may be seen to have swollen and conspicuously protruding apices, while in others these apices have burst and their contents mingle with the mass of secretion lying between the epithelium and the peretrophic membrane. Gehuchten(6) first drew attention to this peculiar method of secretion in the larva of *Ptychoptera contaminata*, but it has since been found to occur in many insects.

Slightly posterior to the cardia the epithelium of the stomach shows a ventral thickening. The degree of development of this ventral thickening varies considerably in different larvae, but I have not been able definitely to correlate its degree of development with the degree of maturity of the larva. The cells of the thickening usually exhibit considerable vacuolation, the individual vacuoles being small. In an example which had a very prominent ventral thickening, examination with an oil immersion lens showed what appeared to be minute branching ducts opening into the gut lumen through the peretrophic membrane which is here closely applied to the surface of the epithelium. It seems probable that the ventral thickening is of a secretory nature. The epithelium of the anterior and posterior sixths of the stomach does not form globules of secretion. The malpighian tubes open into the posterior end of the mid-gut and not into the anterior end of the hind-gut. The same exceptional condition occurs in *Ptychoptera* according to Gehuchten(6) and in the *Meloidae* according to Beauregard(1). The relation of the malpighian tubes to the gut will be considered later. The posterior end of the mid-gut epithelium is continued for a short distance into the anterior end of the hind-gut forming the Pyloric Valve. Small imaginal cells (*i.c.* Fig. 10) occur scattered irregularly along the mid-gut between the bases of the epithelial cells. They are usually placed singly, but occasionally two or three occur together in a small clump.

The hind-gut extends in a practically straight line from the anterior region of the eighth body segment to the anus and is divisible into three regions, an Ileum extending back to the middle of segment X, followed

by a Colon extending back nearly to the middle of segment XII, and terminating in a short Rectum which opens ventrally by the anus. The epithelium of the anterior end of the ileum is folded inwards to become continuous posteriorly with the mid-gut epithelium which is continued into the cavity of the ileum. This inner layer of the epithelium of the ileum consists of imaginal cells which proliferate at the time of histogenesis to form the imaginal hind-gut. The wall of the ileum consists of a well-marked epithelium (except in the extreme anterior region where it is very thin) and two layers of muscles, a thin inner layer of isolated longitudinal fibres and a continuous and rather thick outer layer of circular fibres. In the extreme anterior end the intima is raised into small papillae. In the remainder of the ileum the intima, and with it the epithelium, is raised up into four folds, two large lateral, a small ventral, and a small dorsal. The colon differs from the ileum in having no longitudinal muscles and its circular muscles are in the form of separate thick bands placed at small intervals round the gut. The lateral folds of the intima and epithelium are unchanged, but there are two small dorsal and two small ventral folds. The chitinous intima, particularly on the lateral folds, is produced into blunt spines. The Rectum is a terminal dilatation of the colon. Its epithelium and intima are folded in a complicated manner and the latter is produced into prominent spines pointing posteriorly. No longitudinal muscles are present and the circular muscles are similar to those of the ileum.

The Salivary Glands (Text-fig. 9) are of a peculiar type. Their anterior ends lie dorsal to the first thoracic ganglion, near the median line. From here they extend postero-dorsally as lobed strap-like bodies to the anterior region of the cardiac division of the stomach when they narrow considerably, turn backwards dorso-lateral to the gut and extend back into the first abdominal segment. Their anterior ends are irregularly circular in outline and composed of large cells surrounding a central duct which is a direct continuation of the salivary duct. The middle region of the gland is only one cell thick and is irregularly lobed. The narrow dorsal region consists of only two cells in any cross-section, one dorsal to the other. The cell limits are everywhere very indistinct owing to the fusion of the cells. The central duct extends a short distance into the gland from its anterior end, but beyond its termination there is no central cavity about which the cells are arranged. The secretion is collected from the cells and poured into the main duct by a series of minute ducts. A few of these (*d*.1) appear, at all events in parts of their course, to be intercellular, lying between two adjacent cells. It is difficult to be certain on this point

owing to the impossibility of defining the cell limits. The majority of the collecting ducts are, however, undoubtedly intracellular and appear in cross-section as minute, circular vacuoles in the cell protoplasm, lined by an exceedingly delicate, possibly chitinous, membrane (*d.2*). The cells of the gland are large and much vacuolated. They contain a large spherical nucleus provided with a prominent rounded nucleolus and a considerable amount of chromatin.

Each gland gives off a short duct anteriorly which passes forwards to one side of the connective joining the sub-oesophageal and first ventral ganglia, joining with its fellow of the opposite side immediately in front of this connective to form a median salivary duct which runs forwards to open into the posterior wall of the mouth. The salivary duct consists of a thin non-glandular cellular sheath and a chitinous lining which shows spiral thickenings.

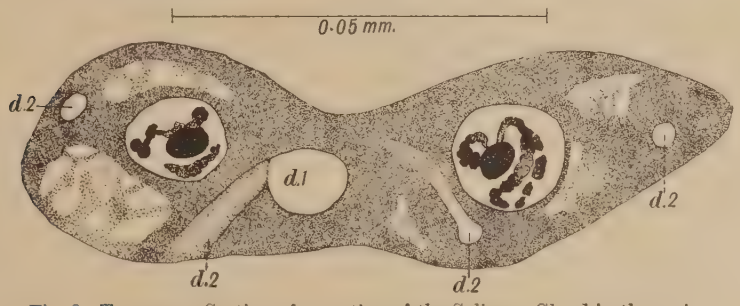

Fig. 9. Transverse Section of a portion of the Salivary Gland in the region of the Cardiac Valve.

So far as I am aware no previous case has been recorded among Dipterous larvae of salivary glands which discharge their secretion by means of intracellular ducts. In *Gli Insetti* (1, 516) Berlese (3) gives a figure of the labial salivary glands of *Mytilaspis*, the cells of which discharge by means of intracellular ducts, but this is the only instance of such glands which I have seen. In the vast majority of insects the salivary glands are hollow vesicles or tubes with a lining layer of secretory epithelial cells discharging into a central lumen, which is continuous anteriorly, directly or indirectly, with the salivary duct.

The Malpighian Tubes are seven in number, four of them being anteriorly directed and three posteriorly. The posterior end of the mid-gut shows a slight dorsal thickening of the epithelium and from each side of this arises a main malpighian tube trunk. The main trunk is short and from its apex arise three dorsal malpighian tubes, two anterior and one posterior.

The two anterior malpighian tubes of each side extend forwards into the posterior region of the third thoracic segment; along their whole extent they are close together, and in places are quite contiguous for a short distance and appear to be united. At intervals they come into close relation with the gut wall and are apparently attached to its sheathing membrane. The two tubes of one side never come into this close relation with the gut at the same point. The anterior tubes show undulations, but no convolutions or sharp bends. The two posterior dorsal malpighian tubes are directed straight backwards and extend to about the posterior limit of the eleventh body segment. They lie dorso-lateral to the gut and do not come into close connection with it at any point. From the ventral region of the posterior end of the mid-gut arises a single ventral posterior malpighian tube. It runs back in the mid-ventral line below the gut as far as the tenth segment. It then bends across to the right, turns forwards and then upwards until it is close to the right posterior dorsal malpighian tube, when it turns backwards parallel to the latter, the two ending at about the same level.

The malpighian tubes are all similar in structure. Each consists of a row of large, approximately cylindrical cells placed end to end. There is a continuous intracellular lumen traversing the whole series. The nuclei are large and rich in chromatin. The shape of the lumen varies somewhat, being sometimes circular, sometimes rayed in cross-section.

The Central Nervous System consists of supra- and sub-oesophageal ganglia, united by oesophageal connectives, and ten ventral ganglia. The supra-oesophageal ganglion is a bilobed mass, the two lobes being united anteriorly by a broad transverse commissure. The posterior ends of the two lobes are separate. Antero-laterally each lobe gives origin to a very short oesophageal commissure which passes forwards and downwards to unite below the oesophagus, with the posterior end of the sub-oesophageal ganglion. Thus the sub-oesophageal ganglion is more anteriorly situated than the supra-oesophageal, and extends as far forwards as the anterior limit of the first thoracic segment. The oesophageal commissures are broad and very short and are hardly distinguishable from the general mass of the brain.

The ventral ganglia are distributed as follows:

1st	Ganglion.	Posterior limit of first body segment.
2nd	,,	Middle of second segment.
3rd	,,	Anterior third of third segment.
4th	,,	Anterior limit of fourth segment.
5th	,,	,, ,, fifth ,,

6th	Ganglion.	Anterior limit of sixth segment.
7th	,,	,, ,, seventh ,,
8th	,,	,, ,, eighth ,,
9th	,,	Between segments eight and nine.
10th	,,	Anterior third of ninth segment.

Between the 1st and 2nd ganglia there are two widely separated connectives. Between ganglia 2 and 3 the connectives are also double but closely approximated; the remaining connectives are apparently single. The ganglia do not vary greatly in size; the seventh is, however, the smallest and the tenth the largest. The tenth ganglion gives off five large posteriorly directed nerves. All ganglia consist of a fibrous tract surrounded by nerve cells which are mainly concentrated ventral to the fibrous tract.

The sub-oesophageal ganglion gives off three nerves on each side from its anterior end, the two dorsal nerves being the largest. Each dorsal nerve goes to a pharyngeal ganglion situated close to the lateral wall of the pharynx a short distance behind the mouth (Text-fig. 13 A, *p.g.*). On each side one of the ventral nerves goes to a small ganglion situated on the Labial Imaginal Disc (Text-fig. 13 C, *l.g.*). The other two ventral nerves appear to be distributed to the ventral hypoderm of the head and some of the cephalic muscles. The above two pairs of ganglia, and a median ganglion, which is described in connection with Weismann's Ring, belong to the stomato-gastric system.

The Dorsal Vessel is a long tube situated slightly dorsal to the gut and extending from the anterior region of the twelfth body segment to the posterior region of the head. In the twelfth segment an arched membrane separates off a dorsal pericardial chamber from the remainder of the segment, the chamber being narrow anteriorly and widening out posteriorly. The posterior end of the dorsal vessel projects backwards for some distance into the pericardial chamber, in the extreme anterior end of which the dorsal and ventral walls of the vessel fuse with the dorsal and ventral walls of the chamber. Thus a cross-section of this region shows three cavities; a central one which is oblong in shape with its long axis vertical, and a lateral one on each side of it and somewhat arc-shaped. The central cavity is that of the dorsal vessel, the lateral cavities are the anterior regions of the pericardial chamber. As it passes backwards into the pericardial chamber, the diameter of the vessel diminishes rapidly and its narrow posterior end is quite free from the pericardial walls and opens into the chamber. I have been unable to find any ostia in the dorsal vessel and the method of "blood" propulsion appears to me to be as follows. If one supposes the pericardial chamber and the

dorsal vessel to contract simultaneously, the blood in the chamber will be forced into the anterior regions of the chamber which lie one on each side of the posterior prolongation of the vessel. The distension of these lateral chambers will tend to compress and close the posterior end of the vessel. Contraction of the vessel will now force its contained blood forwards into the head. On relaxation of the vessel and the pericardial chamber the low pressure in the former will tend to draw blood into it from the latter. As the posterior end of the vessel projects freely into the pericardial chamber and has a very small opening, I think it improbable that blood can be actively forced from the chamber into the vessel.

Posteriorly the dorsal vessel is wide and circular in cross-section, but it narrows considerably and becomes dorso-ventrally flattened as it passes forwards. In the first thoracic segment it traverses the ganglionic mass, lying immediately dorsal to the oesophagus. Delicate strands, which appear to contain no nervous elements, pass from the sheathing membrane of the oesophageal commissures and fuse with the wall of the vessel. In front of the ganglionic mass its shape in cross-section is that of a rather narrow isosceles triangle with its base lying on the dorsal wall of the oesophagus. It retains this shape to a point slightly posterior to the pharynx where it opens freely into the head cavity. The part of the vessel lying in the head and thorax is not provided with pericardial cells and may perhaps be distinguished as the Aorta, though there is no true morphological distinction between it and the remainder of the vessel. The wall of the heart is composed of circular striated muscle fibres whose nuclei occur in the lateral walls in which they form conspicuous bulges (Text-fig. 10, *h.nu.*).

The Pericardial Cells (Text-fig. 10) occur in pairs, one on each side of the dorsal vessel in segments IV to XI. The pairs of cells are separated by considerable intervals and lie upon the dorsal parts of the alar muscles (*a.m.*) with which their protoplasm appears to be continuous, as is the case in the Blow-fly larva according to Lowne (14). There is thus a pericardial cell in connection with each alar muscle, and this arrangement is very common and possibly universal in Dipterous larvae. Pantel (19) in *Thrixion Halidayanum* and Imms (9) in *Anopheles maculipennis* find that on approaching the dorsal vessel the alar muscles split into a dorsal and a ventral layer of fibres, the pericardial cells lying between the two layers. I have myself found a precisely similar arrangement in the larva of *Chlorops taeniopus*. In *Forcipomyia*, however, this splitting does not occur and the alar muscles are inserted into the ventral wall of the heart only. I have been unable to trace the alar muscles to their origin which

is probably in the dorso-lateral body wall. They are probably striped muscles, but none of my preparations shows this clearly. It is possible that at the time when the larvae were fixed the alar muscles had commenced to degenerate in preparation for histolysis, and their staining properties had consequently been altered.

Each pericardial cell contains three or four nuclei (*p.nu.*), but there is no corresponding indication of cell division. The nuclei are rather small and contain a conspicuous nucleolus. Towards the end of larval life (which

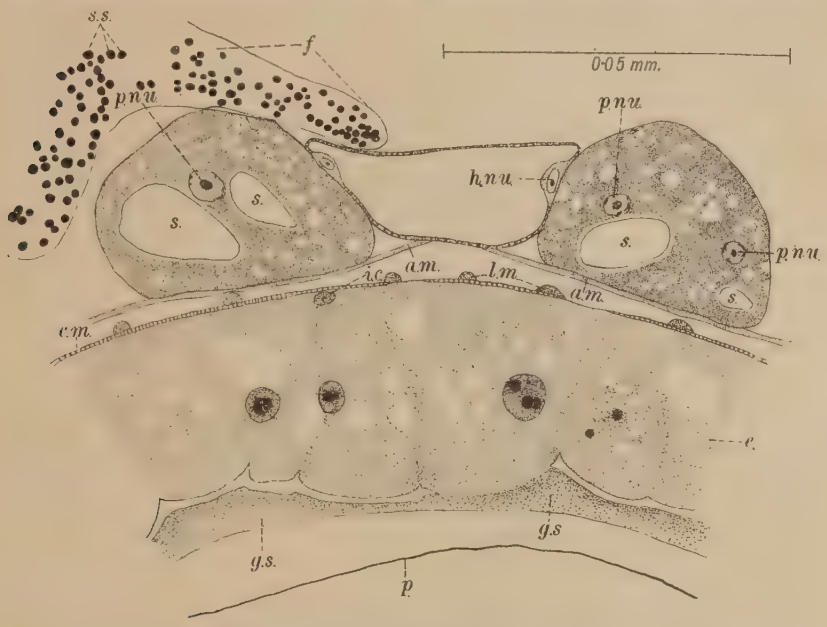

Fig. 10. Transverse Section through the dorsal wall of the Gut, the Dorsal Vessel and the Pericardial Cells. Posterior Region of Mid-gut.

is the stage shown in Fig. 10) the pericardial cells become much vacuolated, and some of the larger vacuoles may be seen to contain a homogeneous body of very regular form (*s.*). Kowalevsky (12, 13) has proved that the pericardial cells have an excretory function, and it seems probable that the homogeneous lightly staining inclusions are composed of waste products extracted from the blood.

In many, if not all, *Muscid* larvae there are ventral "pericardial" cells usually in the form of a transverse loop of cells which passes below the gut, its ends being in intimate relation with the anterior ends of the salivary glands. Such a strand is absent in *Forcipomyia* and has not, so

far as I am aware, been recorded in any *Orthorrhaphous* larva. There are, however, certain cells (Text-fig. 13 A, *c.c.*) which will be mentioned in connection with the labial imaginal disc, which may possibly be of the same nature.

Considering the intimate relation between the dorsal pericardial cells and the alar muscles it is not, I think, improbable that the former are muscle cells of which the alar muscles are part, and that they have become considerably hypertrophied and specialized for an excretory function. Very careful embryological work is of course necessary to settle this point. The following passage from Lowne (14) (p. 343) dealing with the fate of the pericardial cells during the metamorphosis of the Blow-fly larva does, however, give some support to this theory. He says: "The persistent pericardial cells are probably the elements from which the alar muscles of the pericardium of the imago are developed, as I find them embedded in the substance of their fibres."

Weismann's Ring is here in the form of a straight solid cellular rod. Each lobe of the dorsal ganglion gives off from the mesial face of its posterior end a small mass of nervous tissue from the base of which a nerve passes vertically downwards close to the lateral wall of the dorsal vessel, the two nerves uniting to form a small ganglion lying between the ventral wall of the heart and the dorsal wall of the oesophagus, slightly posterior to the oesophageal commissures. I consider this to be the median ganglion of Lowne (14). The two mesially directed nervous masses mentioned above fuse together in the median line between the posterior lobes of the dorsal ganglion, and from their point of junction Weismann's "Ring" proceeds backwards as a thin solid rod of small cells. This rod extends back as far as the cardiac region of the gut, lying, except its anterior end, on the dorsal wall of the heart. In transverse section its anterior region shows more or less distinct indications of consisting of right and left halves. It gives off no strand to the wall of the cardia as does the homologous structure in *Cyclorrhaphous* larvae, from which it also differs in being a solid rod instead of a ring surrounding the dorsal vessel. It is composed of very small cells similar to the cortical cells of the various ganglia. The cell limits are indistinct.

The Fat Body (Figs. 10, 11 and 12, *f.*) varies in extent according to the age of the larva, being most extensive in mature larvae. In all stages it is entirely absent from the head. In a larva killed about the middle of January its general distribution is that of a rather thin and almost continuous sub-hypodermal layer giving off lobes between the various organs. In the abdominal segments it is mainly concentrated into two ventro-lateral masses.

The fat body cells are large and the cell limits are for the most part distinct. The cells contain very numerous small spheres (Fig. 10, *s.s.*) of a homogeneous substance, which stains dark blue with Heidenhain's Iron Haematoxylin. These are probably proteid storage products resulting from the digestive metabolism. In sections stained with Heidenhain's Iron Haematoxylin the nucleus is usually completely masked by the blue spheres; Erlich's haematoxylin, however, does not stain the spheres and the nucleus may easily be examined. The nuclei are spherical and

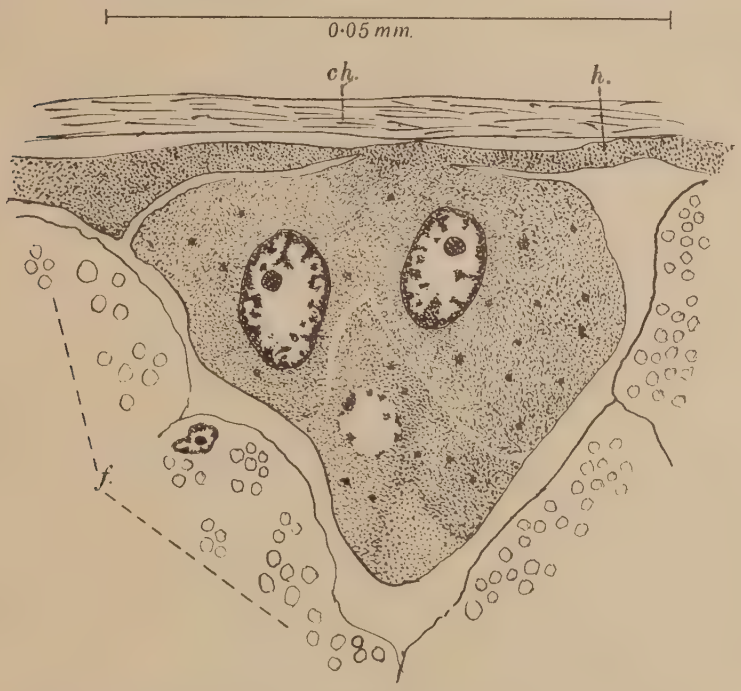

Fig. 11. Transverse Section of Larval Oenocytes. *ch.*, chitin; *h.*, hypoderm.

rather small and contain a prominent nucleolus; their chromatin is mainly peripheral in position. The protoplasm is difficult to study on account of its poor affinity for stains. It appears to be in the form of trabeculae surrounding numerous vacuoles.

Large Oenocytes (Fig. 11) occur in groups in segments IV to XI. According to Mme Hufnagel(7) there are in *Hyponomeuta padella* two sets of Oenocytes, larval and imaginal. The former degenerate during metamorphosis, and the latter develop during the last larval stadium. Although I have only examined the last larval stage of *Forcipomyia*, I

believe that here also there are larval and imaginal oenocytes as I have found what appeared to be the last stages of degeneration of the larval oenocytes close to the positions occupied by the oenocytes to be described. If this view is correct the bodies discussed below are the imaginal oenocytes.

On each side of segments IV to XI inclusive there is a mass of four large cells close to, and connected with, the hypoderm, in the ventro-lateral region of the segment (Fig. 11). During the pupal period they become separated from each other and from the hypoderm and take up various positions in the body. Their protoplasm is densely staining (with Eosin or Lichtgrün), non-vacuolated and rather homogeneous in appearance, but has a few small granules scattered through it. The nucleus is, except for its larger size, very similar to a hypoderm nucleus. It contains a large nucleolus and a considerable amount of chromatin mainly aggregated round the periphery of the nucleus. It seems fairly obvious that the oenocytes are special developments of certain hypoderm cells. This is shown in Fig. 11.

A few *Small Oenocytes* are present scattered through the body. They are not, however, nearly so numerous as they are in *Chironomus* according to Miall and Hammond (18) and in *Anopheles* according to Imms (9).

The Tracheal System is entirely closed. Perris (21) describes the larva of *Ceratopogon* (= *Forcipomyia*) *Laboulbeni* as having nine pairs of spiracles, one on each side of the prothorax and first eight abdominal segments. Malloch (16) says in his synopsis of the characters of the larvae of the Certaopogonidae "...in the terrestrial forms I am unable to find lateral abdominal spiracles." He does not mention any thoracic spiracles. After a careful examination of living larvae, potash cleared mounts and serial sections, I am convinced that stigmata are entirely absent in the larva of *Forcipomyia piceus*.

Tracheae are, however, present and ramify fairly extensively through the body. There are two main longitudinal trunks situated one on each side dorso-lateral to the gut and close to it, and united by a series of dorsal commissural tracheae. I have not been able to follow out the details of the tracheal branches as the larva is a very unfavourable one for the study of these by transparence. The chitinous intima of the tracheal trunks has no spiral thickening. The presence of a well-developed tracheal system in the absence of either stigmata or tracheal gills is peculiar but by no means unique, many similar instances being supplied, for example, by the parasitic *Hymenoptera* (Seurat (22)). The larva lives in a moist atmosphere and gaseous exchanges probably take place through the thin

chitin of the body wall, the body fluid acting as an oxygen carrier. It is usually denied that the body fluid of insects has any respiratory function except in those *Chronomid* larvae whose body fluid contains haemoglobin. The point has, however, never been definitely proved and it is difficult to conceive how respiration can take place in the larva of *Forcipomyia* unless the body fluid has a respiratory function.

In the first thoracic segment there is on each side a very peculiar tracheal branch (Text-fig. 12) going to the hypoderm at a point slightly postero-lateral to the dorsal seta of its side. Before reaching the hypo-

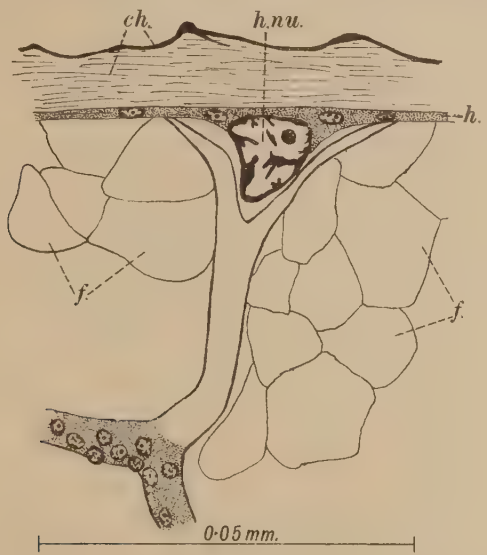

Fig. 12. Section through Vestigeal Stigmatic Trunk in Larval Prothorax.

derm it swells out into a conical ampulla whose base would lie against the hypoderm (*h.*) but has been pushed into the cavity of the ampulla. In this pushed-in base lies a peculiar formation of the hypodermis which consists almost exclusively of a relatively enormous hypoderm nucleus (*h.nu.*). The tracheal trunk which ends in the ampulla has no cellular sheath. This structure is probably to be interpreted as a vestigeal stigma; these have been recorded in *Anopheles maculipennis* by Imms(8) and in *Hypoderma bovis* by Carpenter and Pollard(4). There is no trace of any opening through the chitin (*ch.*).

Integument and Hypodermis. Except on the ventral non-hook bearing region, the body chitin consists of two layers, a thin and dark coloured outer layer and a thicker and lighter coloured inner layer (Text-fig. 12, *ch.*).

The small integumentary hooks consist exclusively of the dark outer layer, and it is this layer which is absent from the ventral body region. The hypoderm (*h.*) is a thin cellular layer, one cell thick, the cell limits being indistinguishable. The oval nuclei form small bulges in the layer. They contain a small spherical nucleolus and their chromatin is mainly peripherally situated.

The dorso-lateral and dorsal setae of the body are lodged over special hypoderm cells which are enormously hypertrophied. Their nuclei differ from those of ordinary hypoderm cells in having the chromatin scattered throughout the nucleus instead of concentrated round its periphery; they contain a large nucleolus. The dorso-lateral setae have a central cavity extending almost to their tips, and this cavity is occupied by an axial protoplasmic filament given off from the basal cell. In the dorsal setae the central cavity extends right to the tip of the seta in the spear-shaped head of which it becomes somewhat enlarged, the whole cavity being occupied by a protoplasmic continuation of the basal cell. It is obviously this cell which secretes the drop of liquid found at the apex of each dorsal seta in the living larva. The large cephalic setae resemble the dorso-lateral body setae. The remaining setae of the body are solid and are seated upon small thickenings of the hypoderm composed of several slightly hypertrophied cells instead of one greatly hypertrophied cell. The dorsal, dorso-lateral and lateral setae of the body are attached to the body wall by distinct articulations, but this does not appear to be the case for the ventro-lateral setae or the minute ventral setae.

The Cephalic Imaginal Discs consist of the rudiments of the imaginal antennae, mouth parts and eyes. Text-fig. 13 A, B and C represents the *Labial* imaginal disc sac as seen in the 30th, 32nd and 35th of a series of transverse sections. The imaginal sac arises in two lateral halves from the ventral hypoderm of the head and projects forwards below the pharynx. In its posterior region each lateral half is a hollow, approximately cylindrical body with its median wall much thickened (C). The salivary duct (*s.d.*) lies between the two halves. There is a small Labial Ganglion (*l.g.*) on the dorsal surface of each half. Anteriorly the two halves unite to form a single hollow sac which is U-shaped in cross-section and has its dorsal wall much thickened (B). The salivary duct lies in the concavity of the U. As it approaches the mouth the sac decreases in diameter (A) and finally ends blindly within the posterior region of the larval labium. Slightly behind its anterior end it receives a nerve from each of the two Pharyngeal ganglia (*p.g.*). At this point there is a transverse row of four large cells (*c.c.*) lying between the labial disc and the ventral wall of the

pharynx. These have some resemblance to the dorsal pericardial cells (they do not resemble those shown in Text-fig. 10 which is from a section of a very late larval stage after the commencement of histolysis; Text-fig. 13 is from a much earlier stage), and it is possible that they represent the ventral pericardial cells of the *Muscidae* though these occupy an entirely different position. Feeding experiments with colouring matters similar to those used by Kowalevsky (12) in his experiments on *Muscid* larvae, can alone determine whether these cells are or are not ventral pericardial cells.

It is evident that the Labial disc as described and figured here is an immature stage, and really consists only of the imaginal sacs, the imaginal discs having not yet formed except as mere thickenings of

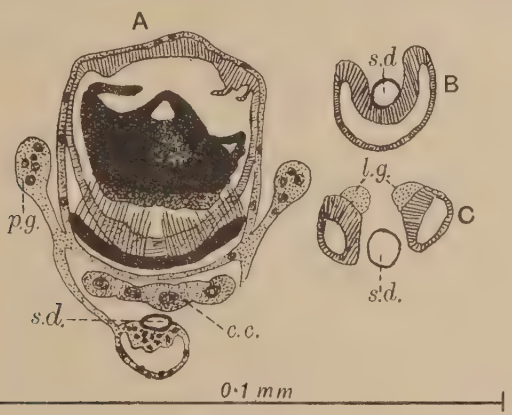

Fig. 13 A, B and C. The Labial Imaginal Disc as seen in the 30th, 32nd and 35th of a series of transverse sections 8μ in thickness.

the wall of the sacs. I am not able at present to give an account of the later development.

The *Maxillary* discs are at first mere thickenings of the hypoderm in the base of the larval maxillae, but later they become true imaginal discs with peripodal cavities and membranes. The disc of the *Labrum* is possibly represented by a region on its dorsal surface where the hypoderm nuclei are crowded together. I have found no trace of *Mandibular* imaginal discs.

Text-fig. 14 A to F represents the 39th, 45th, 47th, 51st, 55th and 61st of a series of transverse sections through the imaginal sacs and rudiments of the antennae and eyes; A and B only show the sac of the right-hand side; C to F show both sacs; C is from a section through the extreme anterior end of the prothorax.

On each side of the head a deep dorso-lateral invagination of the hypoderm (*d.i.*) runs backwards from the base of the larval antennae (A). In places the two sides of the invagination are fused together so as to appear as a single hypoderm lamella. The base of the invagination swells out and constitutes the peripodal membrane (*p.m.*) of the Antennal Imaginal Sac through which runs the antennal rudiment (*a.*). The larval eye lies close to the lateral wall of the invagination but is entirely outside the cavity of the latter. The dorso-lateral invagination does not extend to the posterior border of the head though its ventral enlarged region is continued backwards into the thorax. Thus a transverse section through

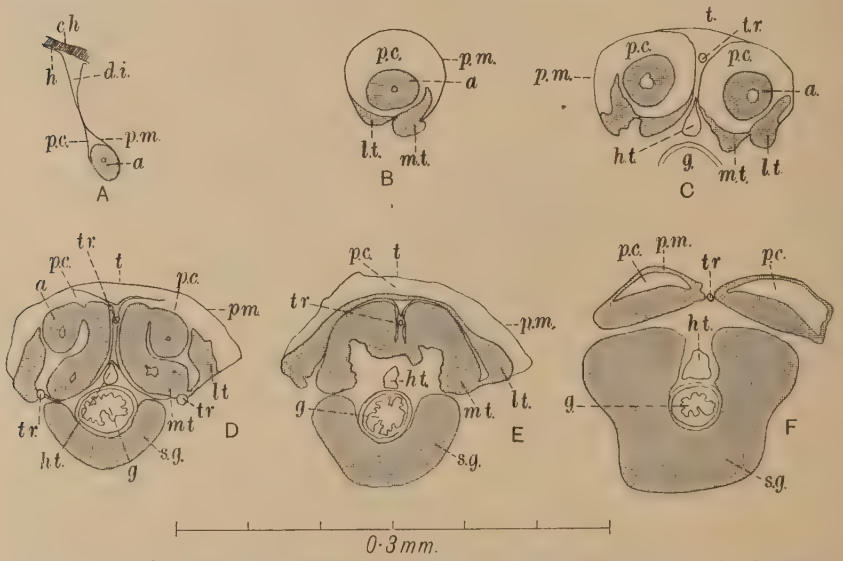

Fig. 14 A–F. The Imaginal Rudiments of the Eyes and Antennae as seen in the 39th, 45th, 47th, 51st, 55th and 61st of a series of transverse sections 8μ in thickness. *g.*, oesophagus; *tr.*, tracheal vessel; *h.t.*, dorsal vessel. For other lettering see text.

the posterior region of the head (B) shows the two imaginal antennal sacs with no apparent connection with the hypoderm of the head. In the anterior region of the prothorax (C) the two imaginal sacs lie dorsal to the gut, almost touching each other in the median line. Dorsally they are joined together by a transverse plate of hypoderm (*t.*) which is derived from the dorsal hypoderm at the junction of head and thorax and extends backwards into the prothoracic segment. In *Chironomus*, according to Miall and Hammond (17), there is, instead of a plate, a hypodermal fold enclosing a narrow space between its dorsal and ventral walls and extending back into the thorax, and the dorso-lateral invaginations of the cephalic

hypoderm extend right to the posterior border of the head and then backwards into the prothorax as invaginations of the ventral wall of the transverse fold. The transverse plate of *Forcipomyia* is probably a reduction of an original fold similar to that in *Chironomus*. In B and C may be seen two thickenings of the ventral wall of the imaginal sacs; these probably form certain regions of the imaginal head capsule in the neighbourhood of the eyes and antennal bases. The more median thickenings (*m.t.*) are directly continuous posteriorly with the bases of the imaginal antennae, the more lateral ones (*l.t.*) with the imaginal eye rudiments. Slightly anterior to the sub-oesophageal ganglion (*s.g.*) the median walls of the two sacs fuse together forming a thin vertical partition of imaginal cells.

At about the level of the anterior end of the sub-oesophageal ganglion the median part of the dorsal wall of the conjoined sacs loses its connection with the lateral regions of the walls (D). At this point the two sacs communicate with each other dorsally and are roofed over in the median region by the transverse plate of hypoderm mentioned above. Slightly more posteriorly the lateral thickenings of the ventral wall of each sac extend upwards as two plates of imaginal cells which unite in the median line immediately above the imaginal antennal bases which are thus excluded from the peripodal cavity (E). This is almost the posterior limit of the antennal rudiments, and it is at this point that the antennal nerves enter their bases. The peripodal cavity (*p.c.*) is now in the form of a transversely crescentic space whose thickened ventral wall is formed by the dorsal extensions of the lateral thickenings of the antennal imaginal sacs. This crescentic space is the Imaginal Sac of the Eye rudiments. Slightly anterior to the supra-oesophageal ganglion it becomes divided into right and left halves by the fusion of its dorsal and ventral walls in the median line. Each half consists of a peripodal cavity enclosed by a thickened ventral wall (which is the eye rudiment) and a thin and arched dorsal wall.

As may be seen from the figures the antennal rudiments extend as straight and thick-walled hollow cylinders through the whole length of the antennal sacs, their apices lying in the bases of the larval antennae. A nerve (not shown in the figures) runs through the axial cavity. In *Chironomus* the similarly disposed nerve is, according to Miall and Hammond (17), the larval antennary nerve, and is replaced in the imago by new nervous elements. I have not followed the later history of this nerve in *Forcipomyia*.

The Trunk Imaginal Discs consist of four in each thoracic segment

and a median ventral one in the last abdominal segment. The points of invagination of the Leg discs are situated slightly posterior to the ventro-lateral setae, and just over each point of invagination is a group of three minute setae already mentioned as the vestigeal legs of the larva. The imaginal sac lies dorsal to its point of invagination, close against the hypoderm of the lateral body wall, and the leg rudiment arises as an ingrowth from the median wall of the sac.

In the mesothorax the imaginal sacs of the Wings are situated dorsal and slightly posterior to the mesothoracic leg sacs. They are dorso-ventrally elongated and lie close against, and their outer wall apparently fused with, the lateral hypoderm of the segment, their point of invagination being at their dorsal ends. Ventrally their peripodal cavity communicates with that of the leg sac. The wing rudiments arise as a ventrally directed infolding from the dorsal wall of the sac. Both leg and wing rudiments have a central cavity. That of the leg persists in the imago and contains the muscles and nerves, etc., of the leg; the cavity of the wing rudiment is only represented in the imago by the lumina of the wing veins. In the metathoracic segment the sacs of the halteres occupy a similar position and are of a like nature to the sacs of the wing discs.

In the prothorax there are two small discs in the positions occupied in the mesothorax by the dorsal ends of the wing sacs. These discs, which are the rudiments of the pupal respiratory trumpets, are at first mere lenticular thickenings of the hypoderm. Shortly before pupation they develop into hollow tubular *outpushings* of the hypoderm and come to lie between the larval hypoderm and the overlying chitin. They are never at any stage invaginated and have therefore no imaginal sac; they thus differ fundamentally from the other thoracic discs and cannot, I think, in spite of their position, be homologized with the imaginal discs of the wings and halteres.

In the last abdominal segment an imaginal sac is developed from the ventral hypoderm slightly anterior to the anus, and projects forwards below the rectum. It contains a longitudinal imaginal rudiment on each side and these are probably the rudiments of the External Genetalia.

I have not followed out the details of the *Muscular System* or the *Genital Organs*. The latter are easily recognizable in the earliest larval stages which I have examined, and have an uninterrupted development not undergoing the histolysis which affects in varying degrees the other larval tissues.

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FURTHER OBSERVATIONS ON THE RELATION OF THE HEIGHT GROWTH OF TREES TO METEOROLOGICAL CONDITIONS*

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(With 2 Graphs.)

I. Introduction. Several attempts have been made to estimate the influence of variable environmental factors in determining the rate of growth of trees by observing correlations between fluctuations in the rate of growth and corresponding variations in the intensity of the environmental factors. Fluctuation in the annual increments of trees can be observed either by measuring the widths of the annual rings of wood laid down in successive years or, in the case of conifers, by measuring the intervals between the annual whorls of branches; these fluctuations may be compared with weather records from neighbouring meteorological stations.

Various writers who have employed this method claim to have demonstrated correlations between annual increments and the following climatic factors:

(i) Rainfall and snowfall either during, or previous to, the growing period.

(ii) Air and soil temperatures during the previous winter.

(iii) Air and soil temperatures during the growing period.

(iv) Rainfall and temperature during July and August of the previous year.

(i) Henry (6) based his conclusions on observations in the neighbourhood of Nancy after the dry summer of 1893 and showed, for that year, that the annual rings of beech, oak, hornbeam and spruce were remarkably narrow, especially in trees growing on dry soils. Mer (10), also working at Nancy, measured annual rings and height increments of silver fir (*Abies pectinata*) for the years 1885 to 1894. The minimum annual rings were made in 1888 (a cold summer) and 1893 (a dry summer), and he concluded that whereas dry summers reduced both ring thickness and

* See Hiley and Cunliffe (8).

height increment, cold summers reduced ring thickness but not height increment. Bogue (2) measured the ring widths of forty-two trees of fifteen species for the years 1892 to 1904, and though no very definite relationship is visible in his figures, he reached the conclusion that abnormally high or low precipitations were evidenced by broad or narrow rings respectively in the *following* year. Stewart (14) found a general relation between the annual rings of a single oak and the rainfall for the years 1843 to 1893.

The most serious attempts to demonstrate a relationship between rainfall and annual ring thickness were made by Douglass (4, 5) on *Pinus ponderosa* in the dry climate of Arizona, and Huntingdon (9) on *Sequoia gigantea* in California. Douglass (5), p. 66, states that "the accuracy with which the pine trees near Prescott represent the rainfall recorded in that city for forty-three years is, without correction, about 70 per cent. By a provisional correction for conservation of moisture in the soil this accuracy rises to about 82 per cent." The degree of accuracy indicated in this statement is rather obscure.

(ii) A correlation between the widths of annual rings and winter temperature has been demonstrated by Schwarz (13) for *Pinus silvestris* in northern Prussia. Using the mean of thirty-one trees, the ring widths of which for the years 1879 to 1894 were measured by Schwarz, we have calculated a correlation coefficient of $+ 0{\cdot}77 \pm 0{\cdot}07$ with January-March mean air temperature and a coefficient of $+ 0{\cdot}78 \pm 0{\cdot}07$ with the soil temperature at 0·6 m. for the same period. From these figures it appears that in this instance winter temperature was more effective in causing fluctuations in ring widths than all other causes together. This occurred in a region in which the mean air temperature for the months concerned was 0·0° C. and the mean soil temperature 2·2° C. Schwarz ascribed the poor growth during seasons following cold winters to the lateness of the renewal of cambial activity.

(iii) Pearson (11) recorded a negative correlation between annual height increments of *Pinus ponderosa* and May-June temperatures and ascribed it to excessive transpiration during hot years, and Robbins (12) observed a negative correlation between ring widths of oak and May-June temperatures. A correlation coefficient of $- 0{\cdot}57 \pm 0{\cdot}11$ has also been calculated between ring widths in *Pinus silvestris* as measured by Schwarz and May-June temperature, but since the latter showed a correlation coefficient of $- 0{\cdot}47 \pm 0{\cdot}13$ with the January-March soil temperatures it is questionable whether much reliance can be placed on this relationship. The writers (8) found a coefficient of $- 0{\cdot}80 \pm 0{\cdot}07$

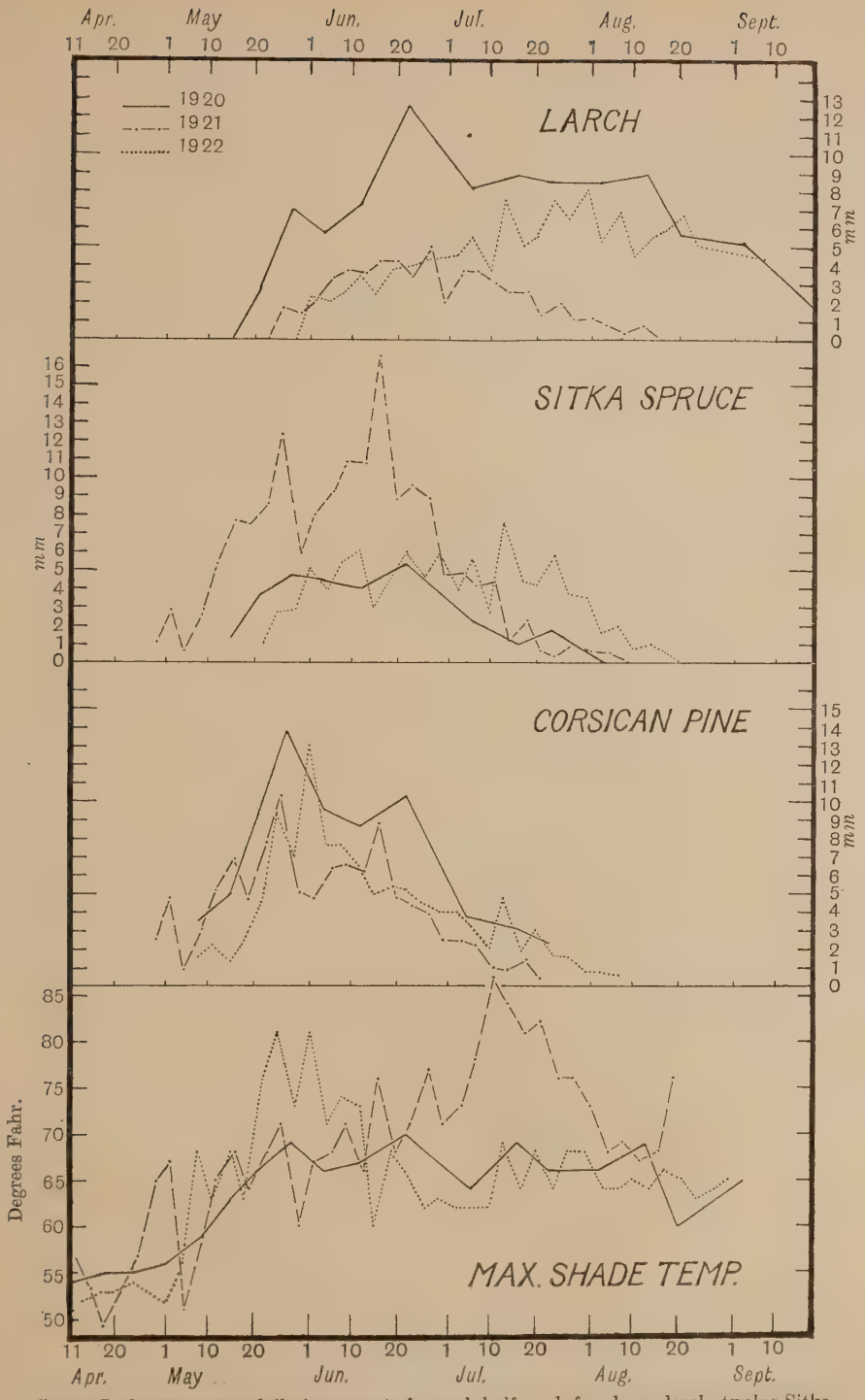
between height increments of Corsican pine at Oxford and May-June soil temperatures at six inches depth, whereas the highest correlation with rainfall, namely that for the period April to June, was only + 0·63 ± 0·10.

(iv) Hesselmann (7) made observations on *Pinus silvestris* in Sweden during the years 1901 to 1903. The year 1901 was abnormally warm and dry, whereas 1902 was cold and wet. The shoot growth in 1902 was 50 to 100 per cent. higher than in 1901 and that in 1903 was abnormally low, facts which he ascribed in each case to the weather conditions during the previous year. Cieslar (3) obtained an apparently contradictory result, for after the dry hot summer of 1904 the height increment of spruce in Austria in 1905 was unusually low. These authors are of opinion that a correlation between annual height increment and the weather conditions in the previous summer can be explained on physiological grounds, since they consider that most of the growth in any year is performed at the expense of food reserves stored up in the previous year[1]. Cieslar considers that the opposite effect of a warm dry summer in the two countries might be due to the fact that Sweden and Austria belong to totally distinct climatic regions. In any case the observations cover periods too short to give reliable evidence and Cieslar's figures do not bear statistical analysis[2]. Cieslar failed to find any corresponding correlation between ring widths and the weather for the previous July and August.

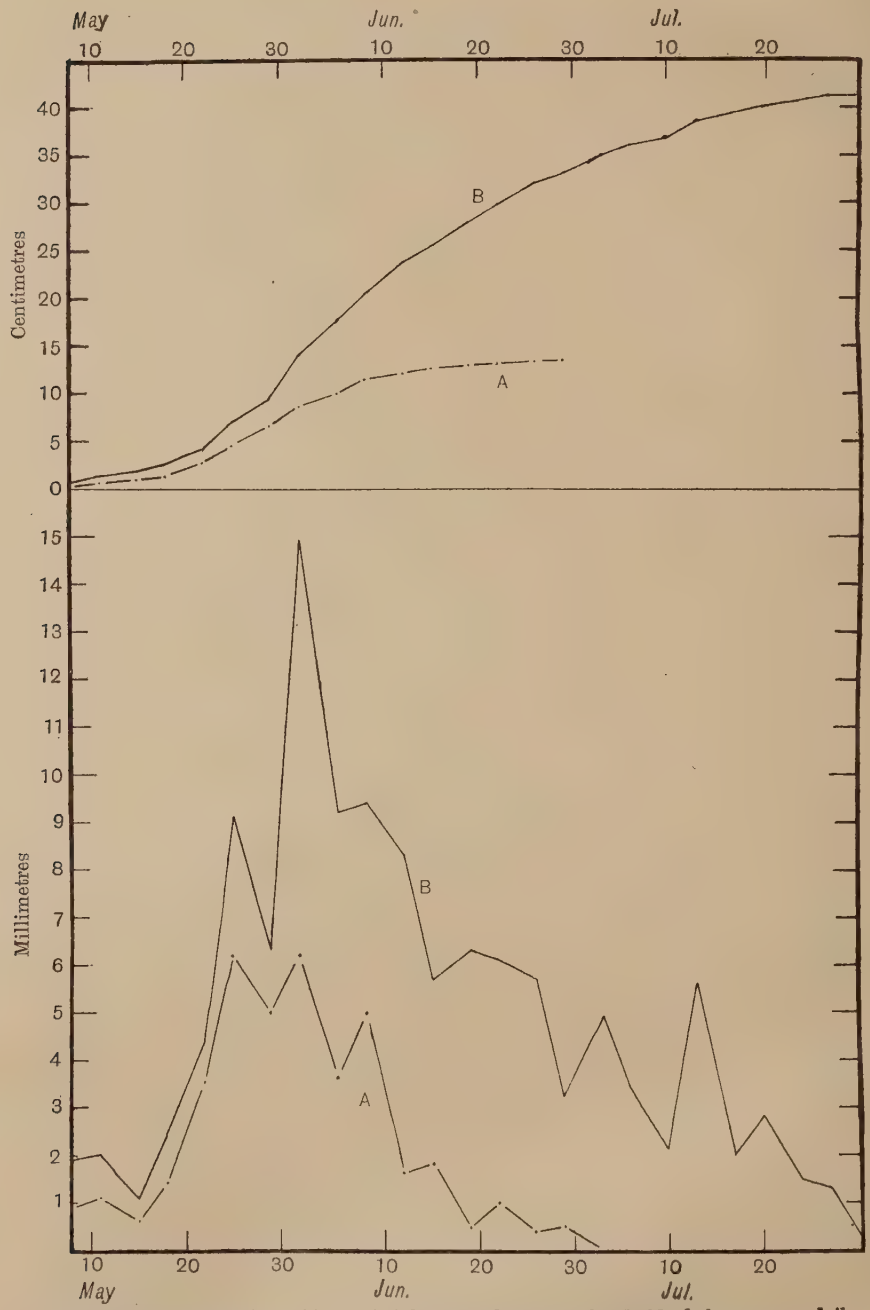
The chief interest in the observations here summarised lies in the fact that with various species in different countries growth is found to depend on very different climatic factors, and in some cases on factors that would not be expected to exert any important influence. This suggests that a statistical study might provide useful information as to the climatic factors which limit the growth of a species in various parts of its range. Such information would be valuable to the silviculturist in deciding what species to plant in given localities, to the ecologist in the study of adaptation, and ultimately to the phytopathologist who will have to take into account the environmental limitations of a species when exploring the possibility of controlling its parasites. At present pathologists are handicapped by ignorance of the specific factors which control the growth of the hosts in different localities. The literature of the genus *Chermes* provides instances of the need for such studies.

[1] See, however, p. 451 of this paper.

[2] Cieslar considers that the expression for climate which most directly affects growth is the "Vegetation quotient" $\left(\frac{\text{precipitation}}{\text{temperature}}\right)$. The correlation coefficient between annual height increment and the vegetation quotient for the previous July–August is only +0·14 for the four years covered by his observations.



GRAPH I, showing mean daily increments for each half-week for eleven larch, twelve Sitka spruce and eleven Corsican pine, together with the mean values for each half-week of the maximum shade temperature.



GRAPH II. *Corsican pine.* Above, height growth curves for 1922; below, mean daily increment for each half-week. *A*, three defoliated trees; *B*, four control trees.

The value of observations of annual increments would be greatly enhanced by a knowledge of the actual period in each year during which the increment was added[1], and the interpretation of such observations may only be possible when we know the direct effect of daily fluctuations in temperature, etc., on the daily increment. In the present paper the half-weekly increments of the terminal shoots of trees belonging to three species (European larch, Sitka spruce and Corsican pine) are recorded, and a comparison is afforded with similar increments for the two previous years already published (8). The results of measurement have been correlated with meteorological fluctuations.

Detailed descriptions of the sites, methods of measurement, etc., have been recorded in the previous paper (8). The same trees were under observation in 1922 as in 1921. Of the trees observed in 1920 only the Corsican pines were on the same site as those observed in the later years; the larch were growing under very similar conditions, but the Sitka spruce were on a drier and less favourable soil.

II. Growth Periods. The mean daily increments for each half-week of the growing season are shown in Graph I. Table I shows the dates of commencement of growth, maximum increment and completion of 80 per cent. of the year's growth. Table II shows the mean monthly temperatures from January to August for the three years, and Table III the monthly rainfalls.

Table I. *Period of Height Growth.*

		Larch	Sitka spruce	Corsican pine
Measurable growth commenced (*i.e.* more than 1 mm. per diem)	1920	May 21	May 15	Before May 4
	1921	May 26	April 28	April 28
	1922	June 1	May 22	May 8
80 % of growth completed by	1920	Aug. 17	June 29	June 29
	1921	July 25	July 11	July 4
	1922	Aug. 17	July 24	July 17
Max. rate of growth	1920	June 22	June 22	May 26
	1921	June 27	June 16	May 25
	1922	July 31	July 13	June 1

Table II. *Mean Monthly Temperatures in Degrees Fahr.*

	Jan.	Feb.	March	April	May	June	July	August
1920	42·1	42·8	46·0	47·2	54·7	58·7	57·5	56·4
1921	45·8	40·4	46·3	47·5	54·4	60·1	68·2	60·9
1922	39·5	40·3	40·4	42·6	57·4	58·4	57·4	56·9

Table III. *Monthly Rainfall in Inches.*

	Jan.	Feb.	March	April	May	June	July	August
1920	2·14	0·63	1·64	3·08	1·69	3·19	4·68	1·11
1921	2·46	0·56	0·93	0·91	1·50	0·34	0·28	1·65
1922	2·42	2·29	1·93	2·83	1·34	1·19	3·55	5·13

[1] Antevs (1) gives a very useful bibliography of publications relating to growth in thickness.

Table IV. *Water Content of Soil*, 1922[1].

Plot	Nature of soil	Depth in inches	Water content in percentage of air dry weight of soil: May 4	May 18	June 8	June 22	July 6	July 20	Aug. 7
Sitka spruce	Clay	0–6	29	28	28	25	28	33	34
„ „	„	12–18	16	26	21	19	24	24	Sat.
„ „	Peaty sand	0–6	130	154	49	?	145	90	160
„ „	„	12–18	Sat.	Sat.	39	19	Sat.	34	Sat.
Larch	Gravel	0–6	34	28	18	5	26	25	39
Corsican pine	„	0–6	17	16	5	5	9	12	—

III. The Relation of Daily Growth to Weather.

(*a*) *Method of analysis of data.* In calculating correlation coefficients the following method was employed.

(i) The logarithms of the mean daily increments for the half-weekly periods were plotted. The mean of each successive set of five readings was taken and these mean points joined by straight lines.

(ii) The differences between the actual logarithms and the values read off from these straight lines were tabulated, the values outside the first and last means being omitted.

(iii) The climatic factors were treated in the same way except that direct values were used in place of logarithmic values.

(iv) Correlation coefficients between the increments for each species and each weather factor were calculated by the formula

$$r = \frac{\Sigma xy}{n\sigma_x \sigma_y}, \quad \text{error} = \pm \frac{0{\cdot}674\sqrt{1 - r^2}}{\sqrt{n}}.$$

(*b*) *Results.* The correlation coefficients are shown in Table V in which the 1921 results are included for comparison. From this table it will be seen that in the case of both Sitka spruce and Corsican pine the results are remarkably similar in the two years. Fluctuations in the rate of growth were again determined principally by maximum or mean shade temperature. The effect of minimum temperature on the growth of Corsican pine appears from the table to be considerable, but no importance should be attached to this since it was found that the cross correlation between maximum and minimum shade temperatures *for the Corsican pine period* is $+ 0{\cdot}48 \pm 0{\cdot}11$.

The larch results for the two years 1921–2 are very different from each other. This may be ascribed to the hot weather in 1921 which, as shown in our previous paper, exerted an adverse influence on the growth of this

[1] The samples were taken from the soil immediately adjacent to the sample trees.

Table V. *Correlation coefficients for* 1922 *compared with those for* 1921 (*the latter in italics*).

Period over which correlation was calculated	Sitka spruce June 1 to July 24	Corsican pine May 15 to July 24	Larch June 5 to Aug. 21	Max. shade temperature June 5 to Aug. 21
Max. shade temp.	+0·66±0·09	+0·72±0·06	+0·54±0·10	—
	+0·73±0·06	*+0·88±0·03*	—	—
Mean shade temp.	+0·65±0·09	+0·76±0·06	+0·58±0·09	+0·81±0·05
	+0·68±0·07	*+0·79±0·06*	*+0·13±0·12*	*+0·91±0·02*
Min. shade temp.	+0·37±0·14	+0·56±0·10	+0·38±0·12	−0·17±0·14
	+0·52±0·10	*+0·47±0·12*	—	*+0·59±0·09*
Diff. between max. and min. shade temp.	+0·42±0·14	+0·16±0·14	+0·25±0·13	+0·85±0·04
	+0·37±0·12	*+0·60±0·10*	—	*+0·60±0·09*
Depression of wet bulb	+0·34±0·15	+0·28±0·13	+0·10±0·14	+0·74±0·06
	+0·20±0·13	*+0·23±0·14*	—	—
Daily horizontal motion of wind	−0·39±0·14	−0·35±0·13	−0·13±0·14	−0·14±0·14
	−0·29±0·13	*−0·06±0·14*	—	*−0·20±0·13*
Hours of bright sunshine	+0·38±0·14	+0·24±0·14	+0·24±0·13	+0·89±0·03
	+0·37±0·12	*+0·44±0·12*	—	*+0·43±0·11*

species. The correlation coefficient with mean shade temperatures in 1922 was + 0·58 ± 0·09, which agrees very closely with the value of + 0·63 ± 0·11 obtained in 1920. A noticeable feature in 1922 was the comparatively high coefficient which was obtained with minimum shade temperature despite the fact that the latter showed no positive correlation with maximum shade temperature during the period concerned. It appears impracticable to trace any correlation between half-weekly rainfall and growth since the effects of rainfall are so protracted. The soil had become very dry on June 22 (see Table IV) but the subsequent heavy rain does not appear to have influenced growth.

The regression coefficients show that in Corsican pine the rate of growth in 1922 was doubled by an increase in maximum shade temperature of 15·4° F. or mean shade temperature of 13·3° F. as compared with 9·0° F. and 7·0° F. respectively in 1921. For Sitka spruce the corresponding values for 1922 were 13·6° F. and 11·0° F. and for 1921, 10·9° F. and 9·1° F. respectively. This year we have been able to obtain regression coefficients for larch which indicate that the rate of growth doubles with an increase of 20·5° F. in maximum shade temperature or 17·4° F. in mean shade temperature.

IV. Effect of the Artificial Defoliation of Corsican Pine. The food material from which the tree makes its annual increment is doubtless derived partly from the reserve stored through the winter and partly from current manufacture. As a preliminary attempt to distinguish

between the relative value of these two sources three Corsican pines were artificially defoliated towards the end of April. Pines lend themselves to such treatment better than other trees since the leaves of the current year do not emerge from the sheathing scales of the dwarf shoots until the height increment of the shoot that bears them is complete.

In order to prevent undue wounding the old dwarf shoots were not torn off, but the needles were pinched off above the sheathing scales. Basal growth occurred and the needles had to be pinched off again on June 1, 12, 22, and July 3. On June 1 the needles had attained a length of 1 to 2 cms., and although they were then cut short they grew out to about 3 cms. by June 12. Hence it cannot be maintained that carbon assimilation was entirely prevented, though it was probably reduced to less than 10 per cent. of the normal.

The defoliated trees had shown normal growth during the four previous years, the mean annual increment having been 45·5 cms. for these trees and 45·2 cms. for four trees used as controls. However, the 1922 increment of the defoliated trees was 13·4 cms. whereas that of the controls was 41·2 cms. Thus defoliation reduced the increment to 33·3 per cent. As will be seen from Graph II, the two sets of trees started growth at the same time, and until May 29 the defoliated trees maintained a growth of 70·8 per cent. of the controls. After this date the growth of the defoliated trees slowed down very markedly and it was completed nearly a month before that of the controls.

V. Summary and Conclusions. (1) The years 1920 to 1922 show a large range of difference in temperature and rainfall; 1920 was a year of even temperature throughout the summer, 1921 was exceptionally dry throughout, and particularly hot in July, 1922 was exceptionally hot from May 21 to June 2 but subsequently resembled 1920. The trees that have been observed have been affected to very varying extents by these meteorological differences. Corsican pine behaved very similarly in each year, and its growing period may be regarded, in the neighbourhood of Oxford, as being approximately from May 1 to July 25. The tree appears to be capable of growing at a rate of 7·5 mm. a day at any time from May 20 to June 27 if weather conditions are favourable.

After the cold spring of 1922 Sitka spruce began growth nearly a month later than in 1921. A rapid growth of 7·5 mm. a day or more has been recorded at various dates between May 16 and July 13.

Larch grows, under favourable conditions, all through the summer, and rapid growth may be observed from the end of May to the end of August.

The results recorded in this paper show that caution is necessary in calculating correlation coefficients between annual increments and the weather during the growing periods owing to the variability of these periods. In general, May and June cover the active period for Corsican pine with fair accuracy, but the former month may, or may not, be significant for Sitka spruce, and in the case of larch, the weather during August and September may, or may not, be of importance in determining the annual increment.

(2) The measurements made in 1922 confirm the conclusion previously reached that temperature is the dominant factor in determining the daily fluctuations in growth. In 1921 this fact was obscured in the case of larch, apparently because growth was repressed by the very high temperature and low rainfall during the growing period. Corsican pine and Sitka spruce were less sensitive to temperature in 1922 than in 1921 as is shown by the fact that in 1922 it took a greater increase in temperature to double the rate of growth than was the case in 1921. Larch is less sensitive to temperature than either Corsican pine or Sitka spruce.

(3) The effect of artificial defoliation on Corsican pine was very marked. The height increment during the year of the defoliated trees was one-third of that of the controls. During the early part of the growing season the growth of the defoliated trees was closely comparable with that of the controls but the rate of increment fell rapidly. Defoliation must greatly reduce both transpiration and carbon assimilation. The fact that growth of defoliated trees was rapid during the early part of the season suggests that the reduction in transpiration was not a controlling factor, and the trees were not greatly affected by mechanical injury. The result is most easily explained on the supposition that the reserve food supplies which maintained growth during the early part of the season became exhausted by July 3. Even before this date the normal growth was dependent more on current manufacture than on reserve food. It thus appears that two-thirds of the annual increment is made at the expense, not of reserve material, but of food manufactured during the season. This result is in direct contradiction to Cieslar's contention cited on p. 444.

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REVIEWS

Practical Bee Anatomy. With Notes on the Embryology, Metamorphoses and Physiology of the Honey Bee. By ANNIE D. BETTS, B.Sc. (LOND.). The Apis Club, Benson, Oxon, England. Pp. 76. 1923.

This little manual forms Volume I of "The Apis Club Library." The object of the series is to provide hand-books on the science and practice of Bee Culture in all its important phases. Apiculture, if it is to attain the status of a highly developed rural industry, needs the bee-keeper to acquire more exact knowledge of his subject and specialisation is also desirable in many cases. With these motives in view the publishers have decided to issue a number of volumes each complete in itself. We may say that the present volume forms a very thorough and exhaustive treatment of bee anatomy. It contains all the information that the most exact and enterprising bee-keeper is likely to demand on this subject. In fact it goes into far more detail than the majority of bee-keepers will attempt to assimilate. The professed biologist might also be well advised to turn to it, for he will find in its pages a thoroughly reliable exposition of bee-morphology with the most important literature quoted in the bibliography. The authoress is evidently well acquainted with the German language, and is able to lay before the reader the chief results of continental researches. The book contains useful notes on dissection, technique, etc., and there are a large number of figures grouped on twelve plates. These illustrations, unfortunately, are poor and sketchy and often difficult to interpret.

Manual of Entomology with special reference to Economic Entomology. By H. MAXWELL LEFROY, M.A., Professor of Entomology, Imperial College of Science. Illustrated by L. N. Stanilard. Pp. xvi + 541. 4 plates and 179 figures. London, Edward Arnold and Co., 1923. Price 35*s*.

In this book the author gives an account of the orders and the great majority of the families of insects, which cannot fail to be of service to students who, having acquired a ground-knowledge of structure and life-history, desire to pursue the systematic branch of the subject in detail. Professor Lefroy tells us in his preface that the volume "is based upon the lectures given as the second of three parts of a course occupying one year of a full training in economic entomology." The students who have received in the form of lectures the instruction comprised in this book, had doubtless, during the first part of their course, examined in the laboratory various insects both in the adult and larval stages, and had been thereby prepared for the professor's systematic teaching. It is to be regretted that the absence of the morphological and developmental details that are essential as an introduction to the study of entomology seriously reduces the value of the book to the elementary student, who,

starting to work through it, will find himself confronted on the first page with a technical description of the Apterygota and a discussion of their affinities—a group of which his preliminary knowledge is likely to be of the slightest. Professor Lefroy admits that he has "dealt lightly with structure and morphology," but he, as an enthusiastic and successful teacher, must realise that some ground-work of general knowledge is an absolutely necessary preparation for the appreciation of the wealth of information here provided for ensuring the further progress of the earnest student. One result of this omission is that the reader is frequently confronted with dogmatic statements about relationships and lines of descent for which no justification is put forward, and from which many entomologists would dissent. Does not the author exaggerate the divergence between Apterygota and Pterygota in view of the close correspondences between a thysanuran and a mayfly larva, and can the Protura, with their heads devoid of feelers and their specialised piercing and suctorial jaws, be regarded as even "possibly the most primitive group" of the Aptera?

Professor Lefroy's plan of exposition is to give a very brief diagnosis, followed by a summarised account of each order and family, mentioning or describing particularly those families and in some cases species which are of economic importance. The information given will be of much value to the appreciative reader, because it comprises many facts not available in ordinary text-books. For example, the sections on the Acridiidae and the Termitidae contain an excellent summary of our knowledge for the student of bionomics and of economic zoology. Perhaps the book would have been more valuable from the latter standpoint had the author contented himself with a very brief reference to families of no economic importance and so found scope to describe more fully those insects which come into direct relation with human health or industry; the Scolytidae, for instance, cannot be effectively dealt with in the two pages allotted to them, and the Oestridae are dismissed in less than a page.

There is no formal bibliography, but references to authors' names with dates will enable a student, by use of the *Zoological Record*, to trace the papers quoted. In some cases, however, he will need to turn to the volume for the year following that mentioned in order to find the reference. The illustrations are clear and to a large extent original; some of them would have been improved in appearance had the drawings undergone more drastic reduction in the process-work. It may be presumed that the author intends to issue later the first and third parts of his course, and thereby afford those students who have not the privilege of his personal instruction an introduction to the whole subject and a detailed discussion of important economic species with directions for checking their ravages.

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